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EXPERIMENTAL RAT CARIES[1]

I. PRODUCTION OF RAT CARIES IN THE PRESENCE OF ALL KNOWN NUTRITIONAL ESSENTIALS AND IN THE ABSENCE OF COARSE FOOD PARTICLES AND THE IMPACT OF MASTICATION

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SIX FIGURES

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Considerable evidence, reviewed elsewhere (Hodge and Sognnaes, '46), indicates that the conventional type of experimental rat caries is initiated by mechanical injury to the teeth caused by the masticatory impaction of coarse food particles. Indeed, 1 author (Rosebury, '39) included all work on rat caries when he concluded that "the appearance of fissure caries among a group of animals, in whatever degree, is definite evidence that such a primary cause was present," and went on to formulate the concept that "dental caries in man is caused primarily by such food," i.e., by the impaction of coarse food particles.

Recent experimental and clinical studies (Sognnaes, '47, '48a, b) were not compatible with such generalization of an oral environmental mechanism. From further experiments with the rat, additional evidence can now be presented to show (1) that rat caries can be produced by a ration which, besides being adequate in known nutritional essentials, is free from coarse food particles; (2) that the carious lesions will develop

[1] The term "rat caries" refers here to the problem of caries production in the ordinary laboratory rat *(Mus Norvegicus)*, and does not include the lesions produced in the newer experimental rodents (cotton rats, Syrian hamsters).

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even if the rat molars are relieved from the impact of mastication; and (3) that the lesions are grossly and histologically comparable to human caries.

EXPERIMENTAL

1. The properties of the caries-conducive purified ration

The nutritional and physical composition of the purified ration is shown in tables 1 and 2, respectively. Nutritionally, this ration is essentially the same as that which, with the addition of ascorbic acid, has been found to produce good growth and health in the rhesus monkey (Waisman et al., '43).

TABLE 1

Composition of the caries-conducive purified ration

COMPONENT	AMOUNT	COMPONENT	AMOUNT
Sucrose, granulated (%)	67	*Vitamins*	
Casein (%)	24	Thiamine (p.p.m.)	3.5
Corn oil (%)	5	Riboflavin (p.p.m.)	3.5
Salt mixture[1] (%)	4	Nicotinic acid (p.p.m.)	25.0
Calcium (%)	0.53	p-Aminobenzoic acid (p.p.m.)	300
Phosphorus (%)	0.55	Pantothenic acid (p.p.m.)	20
Magnesium (%)	0.033	Pyridoxine (p.p.m.)	3.5
Iron (p.p.m.)	150	Inositol (p.p.m.)	1000
Manganese (p.p.m.)	50	Choline (p.p.m.)	1000
Copper (p.p.m.)	15	Vitamin A (U.S.P. Units/gm)	30.0
Cobalt (p.p.m.)	0.65	Vitamin D (U.S.P. Units/gm)	1.5
Potassium (%)	0.45		
Sodium (%)	0.44	Liver concentrate (%)	4
Chlorine (%)	0.76		
Iodine (p.p.m.)	20		
Zinc (p.p.m.)	30		
Sulfur (p.p.m.)	480		
Fluorine (p.p.m.)[2]	2.5		

[1] Modification of Phillips and Hart's salt mixture (J. Biol. Chem., *109:* 657, 1935) by Shaw, J. H. (J. Dental Res., *26:* 47–51, 1947).

[2] This trace element is not added for general nutritional purposes but is due to minor impurities of the carbohydrate fraction and the salt mixture (Shaw et al., Proc. Soc. Exp. Biol. Med., *59:* 89–92, 1945, and personal communication).

In the rodents, the identical purified ration has, in addition, been found to promote good reproduction, which is particularly true of the rat, with which species this and the preceding work with the purified ration (Sognnaes, '47, '48a) is mainly concerned. The present series of rat experiments has been started with a ration adequate in all known essentials. Many

TABLE 2

The particle size of the caries-conducive purified ration

COMPONENTS	QUANTITY	PERCENTAGE DISTRIBUTION OF PARTICLES (gm/100 gm sample)					
		Over 20 mesh	20–40	40–60	60–80	80–100	Below 100
	%						
Granulated cane sugar	67	0.3	39.2	42.4	12.0	3.4	2.7
Casein (and B-vitamins)	24	0	26.0	42.2	12.8	5.2	13.8
Salt mixture	4	2.2	18.1	12.5	4.3	5.5	57.4
Corn oil (and fat soluble vitamins)	5	0	0	0	0	0	(5)
All components	100	0.3	33	39	11	4	7(&5)
Portion requiring mastication	24	0[1]	6	10	3	1	4

[1] This fraction constitutes 60% of the conventional caries producing coarse meal ration, the comparative particle size of which is reported elsewhere (Sognnaes, R. F.: Masticatory efficiency of rats (II), Am. J. Orthod. and Oral Surg., *27:* 383–388, 1941).

factors which are present in natural foods, but whose nutritional importance is still unknown, are absent from the purified ration. This is in contrast to earlier rat caries work where the inclusion of powdered milk, alfalfa, linseed meal, whole corn, or rice particles (coarse rations) no doubt added unknown quantities of unknown trace elements, the confusing presence of which fortunately could be ruled out in this series of studies.

The purified ration has a physical composition such that it literally melts in the mouth. But for the sake of present or future comparisons with other caries producing rations, the particle size distribution has been expressed by actual measurements. It is shown in table 2 that the purified ration is essentially free from particles over 20-mesh and that a total of only 24% of the ration lends itself to some degree of mastication, while no fraction actually requires extensive mastication since it is readily dissolved by or suspended in saliva. The particle size of the previously used coarse caries producing diets has been analyzed elsewhere (Sognnaes, '41). In these, the hard corn or rice particles, too large to pass a 20-mesh screen, represented 60% of the experimental ration (Hoppert, Webber and Canniff, '31). Particles over 20-mesh would correspond to a volume practically as large as the molar crowns of the rat. During the mastication of such a coarse ration, it was therefore not surprising that fractures of the molar cusp ensued, because 20-mesh particles would be too large to be properly masticated by the molars and, on the other hand, too small to be manipulated easily with the rat's forelegs and gnawed upon by the incisors.

Besides the absence of coarse food particles in the purified ration, the rats in the present as well as in the other most recent studies by the author were kept in screen bottom cages. Thereby the possibility is eliminated that accidental tooth fracture and food-impaction might be caused by the rat's mastication of wood shavings, the use of which as bedding has not always been stated but deserves consideration in the evaluation of this type of experiment. The latter precaution as well as the fine consistency of the purified ration would seem to satisfactorily rule out traumatic factors in the production of caries in animals kept under such a regimen. Nevertheless, in view of the wide acceptance in the past of a mechanical factor as the initial cause of rat caries, 2 experiments have been carried out, in which the parts played by the impact of mastication and the physical coarseness of the ration have been completely eliminated.

2. Production of rat caries in absence of mastication and coarse food particles

In both experiments to be reported, representatives of the Long Evans strain of *Mus Norvegicus* were fed the purified ration described above. The carbohydrate fraction consisted of sucrose, in the form of granulated cane sugar, except for 1 series in the second experiment in which the sucrose fraction was replaced by dextrin. For the first experiment (table 3), a litter of 9 rats was weaned at 26 days of age, at which time the animals were transferred from the stock diet [2] to the purified ration for the duration of the experiment. When the rats were 30 days old, the mandibular right molars of each animal were relieved from the impact of mastication by an extraction method described elsewhere (Sognnaes, '41). The first and second right maxillary molars were extracted, while the third molar was left to maintain the occlusal level. In this way is prevented the remote possibility of any "occlusion" between the edentulous part of the maxillary process and the opposing first and second mandibular molars, which are the most caries susceptible teeth in the rat. With these precautions, it was assumed that a reliable comparison could be made between the mandibular right and left first and second molars within each animal with respect to the effect of the degree of masticatory impact on the caries production.

For the second experiment (table 4) 3 litters of 28 rats were used, representing 2 closely related groups which had been maintained on the purified sucrose ration for 1 and 2 generations, respectively. Littermates were divided at weaning, from which time one-half of the rats received the purified diet, and the other half was fed a diet which contained coarse dextrin instead of sucrose as the carbohydrate component of the purified ration. In this experiment, all animals had a full complement of teeth. The results are based upon a comparison of the caries incidence of littermates as revealed by the binocular examination. When sacrificed, the jaws of the

[2] Purina laboratory chow.

animals in both experiments were fixed in 10% formaldehyde, examined under a binocular microscope (30 ×), and prepared for histological study.

In the extraction experiment (presented in table 3) a comparison is made between the distribution of grossly visible carious lesions in the right (experimental) and left (control) mandibular molars. In the 5 animals sacrificed after 75 days

TABLE 3

A comparison of the bilateral distribution of rat caries with and without masticatory impaction of the purified versus a coarse ration

PRE-EXPERIMENTAL PROCEDURE	EXPERIMENTAL		NO. OF CARIOUS AREAS IN LOWER JAW							
			Left molars				Right molars			
	Ration	Period	I	II	III	Total	I	II	III	Total
		days								
Group A (The *right* I and II molars of the upper jaw extracted)	Purified	75	0	0	0	0	0	0	0	0
	Purified	75	0	0	0	0	1	0	0	1
	Purified	75	0	0	0	0	1	1	0	2
	Purified	75	0	0	0	0	1	1	0	2
	Purified	75	1	0	0	1	1	1	0	2
		75	(average)			.2				1.4
	Purified	130	0	1	0	1	1	1	0	2
	Purified	130	1	1	0	2	1	1	0	2
	Purified	130	2	1	0	3 [1]	2	2	0	4 [1]
	Purified	130	2	2	0	4	1	1	0	2
		130	(average)			2.5				2.5
Group B [2] (The *right* I, II and III molars of the upper jaw extracted)	Coarse corn	100	0	1	0	1	0	0	0	0
	Coarse corn	100	0	1	0	1	0	0	0	0
	Coarse corn	100	0	2	0	2	0	0	0	0
	Coarse corn	100	1	2	0	3	0	0	0	0
	Coarse corn	100	1	3	0	4	0	0	0	0
		100	(average)			2.2				0

[1] The carious lesions of this animal are shown in figure 1.

[2] This part is quoted from "Mastication and experimental rat caries" (Part IV) by Sognnaes, R. F., Am. J. Orthod. and Surg., *27:* 552–556, 1941.

on the purified ration, binocular examination revealed no visible carious destruction of the left molars. This finding is in agreement with the previously mentioned work in which rats with a full complement of teeth were fed for a similar period of time after weaning. In the right (experimental) molars, which were without functional occlusion, there were on the other hand 1–2 gross lesions in all but one of the same group of animals and obviously after the same period of time. Consequently, there appears to be an increment rather than a decrease in caries in the absence of masticatory function. In the littermates which were continued on the experiment, the number of carious lesions increased considerably beyond the incidence encountered in non-operated rats with a similar dietary history. After a total of 130 days, the caries incidence became equal and quite marked on both sides of the jaw. Figures 1 and 2 illustrate the very similar amount of carious destruction in the left and right mandibular molars, i.e., those with and without opposing teeth, respectively. The illustrations are from an animal which was marked and compared with its littermates in table 3.

For the sake of comparison, table 3 also records (group B) the distribution of lesions produced in rats fed a coarse corn ration used in an earlier extraction study which was similar except for the diets. On such a coarse ration, the removal of the impact of mastication completely prevented caries (Sognnaes, '41; Ginn, '42) while on the purified ration, the reduced masticatory function appears to have enhanced the initiation of carious lesions. The final bilateral distribution of caries may indeed have been influenced by a general impairment of function of the jaws. But aside from the caries incidence, there was no evidence that the impairment of normal masticatory efficiency affected the ingestion and utilization of the purified ration as far as could be determined by growth and general health.

The second experiment (table 4) shows that the inclusion of a coarser carbohydrate fraction in the purified ration is not caries conducive *per se*. In view of previous data ob-

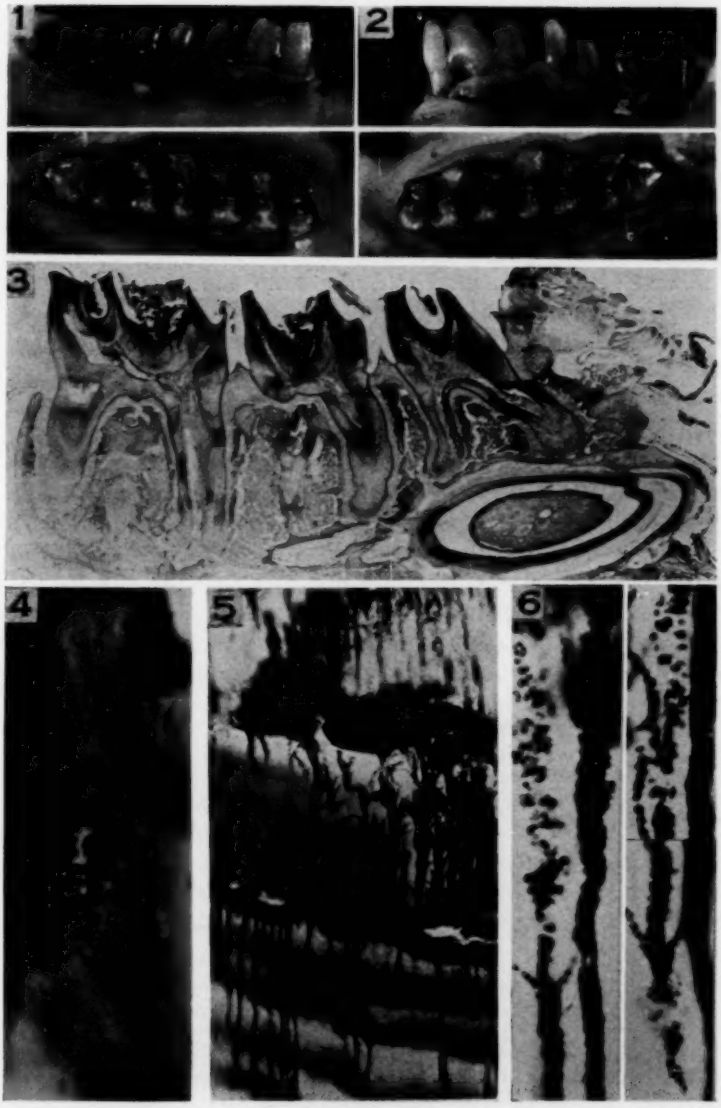
tained with the coarse corn diet, consideration of the physical nature alone would indeed suggest the opposite. In the 15 rats fed the coarser dextrin ration, there was significantly less caries than in their 12 littermates fed the finer sucrose ration for a comparable period of time. This experiment lends further strength to the material presented elsewhere (Sognnaes, '47, '48a), on the basis of which it must be interpreted. There is a much greater cariogenic effect of sucrose than of dextrin when a change from one to the other is made post-eruptively. The great difference in caries in the 2 generations lends further support for a mechanism, operating before tooth eruption, and resulting in an increase in caries with the length of time of maternal maintenance on the purified ration. For our present purpose, it is evident that during such a period of non-function of the teeth, the mechanism of masticatory or traumatic injury of the enamel can have no conceivable direct bearing upon the initiation of caries. From the above experiments it may therefore be concluded that mechanical injury is a minor factor, if any, in the production of experimental rat caries of the nature reported by the writer in this and in the other most recent experiments with the purified ration.

3. Description of the carious lesions

Binocular examination of the carious molars (figs. 1 and 2) reveals a low degree of attrition, presence of undermining cavities and absence of fractures of the cavity walls and cusps. The high cusps are illustrated by the overall view of the section shown in figure 3, which is representative of a 3-month old rat, raised on the purified ration. In contrast to lesions

Figs. 1 and 2 Left (1) and right (2) mandibular molars of rat indicated by footnote 1 in table 3. Top: lingual view, showing absence of fractures and attrition in the non-functioning right first and second molars. Bottom: occlusal view, showing similarity in the extent of carious destruction in the left (control) and right (experimental) teeth.

Figs. 3 to 6. Gram stained paraffin sections, showing occlusal caries in the first and second molars (fig. 3, × 10), spheroid organisms invading the enamel (fig. 4, × 1200), lateral spread in dentin (fig. 5, × 200) and microörganisms filling the dentinal tubuli and their branches (fig. 6, × 1000).



Figures 1 to 6

TABLE 4

Litter mate comparison of molar caries in succeeding generations of rats with masticatory function intact but posteruptively fed the purified fine (sucrose) versus a medium-coarse (dextrin [1]) ration

GROUP	PRE-EXPERIMENTAL HISTORY	POST-ERUPTIVE EXPERIMENTAL PERIOD		CARIOUS SCORE OF MOLARS POST-ERUPTIVELY EXPOSED TO	
				Sucrose ration	Dextrin ration
		days			
I	Transferred from stock diet to purified ration at maturity	130		0	–
II (Litter of I)	Mother as well as litter subsisting on purified ration	100		0	–
		100		1	0
		100		2	0
			(average)	1	0
		130		2	–
		130		2	–
		130		4	0
		130		7	1
			(average)	3.7	.5
III (Litters of siblings of II)	Grandmother as well as mother and litters subsisting on purified ration	100		8	0
		100		8	0
		100		9	1
		100		–	1
		100		–	1
		100		9	0
		100		9	0
		100		12	1
		100		–	0
		100		–	2
		100		–	3
			(average)	9.1	.8

[1] In this ration the sucrose fraction was replaced by dextrin, which after soaking in water was dried and ground to particles, 40% of which were over 20 mesh.

produced by coarse diets, initial lesions in the enamel produced by the purified ration occur without fractures. Even on the lateral wall of the cusps, where fractures are very unlikely, regardless of the ration, there is evidence of early invasion of the enamel (fig. 4). In the described type of lesions, the dentine in the bottom of the cavities is usually very soft, more or less pigmented, and invaded by microörganisms. Following the dental tubuli and their branches, the process eventually spreads laterally causing large decayed clefts in the dentine (fig. 5). Under oil immersion, Gram stained sections reveal a spheroidal type of microörganism, both in the enamel (fig. 4) and in the furthest extension of the lesions into the dentine (fig. 6).

If the described lesions are compared with caries in man, the great similarity is obvious. Undermining cavities, soft pigmented dentine, lateral spread and cleft formation, invasion of the enamel as well as the dentinal tubuli by microörganisms with "pioneer-bacteria" invading the deepest layers, can be regularly demonstrated in the rat caries produced on the purified ration. Counterparts of the above description of the experimental lesions as well as the accompanying illustrations can be found in most textbooks on the histopathology of human caries.

COMMENT

In view of these results, the earlier type of rat caries produced by coarse rations seems to be explained by a coincidental choice of a food particle-size which could not be handled by the rats without mechanical injury to their molars. This does not detract from the importance of Hoppert, Webber and Canniff's work to the development of an experimental approach to the caries problem. Indeed, their method of caries production in the rat has been for over a decade a unique and useful *in vivo* adjunct to other means of elucidating factors influencing the progress of dental caries. As has been shown elsewhere (Hodge and Sognnaes, '46), this is particularly true of studies on the relationship of fluorine

to dental caries. But beside the artificial nature of "fracture caries" itself, the coarse corn rations by which these lesions are produced are composed of a variety of food sources of indefinite and indeed variable composition with respect to the known and unknown essential nutritional factors. In addition, no one essential nutrient can be experimentally varied through a wide range of concentrations independently of all other nutrients. By way of comparison, the composition of the purified ration is standardized and any nutrient can be independently altered at will. Thus, the purified ration permits the examination of the function of specific food factors in the etiology of caries which cannot readily be achieved by a diet composed of natural foodstuffs.

The pattern of caries production in rats fed the purified ration seems more suggestive of a nutritional mechanism operating during tooth development than of a purely physical mechanism operating during function of the teeth. Work is now in progress to test the importance to dental health of food factors not included in the otherwise adequate purified ration. For the present, it is difficult to conceive the nature of a nutritional depletion unless more evidence can be brought forth to show that the teeth are particularly sensitive (at least during their developmental period) to as yet unrecognized nutritional injuries. Therefore, at the present time, it is not possible to explain the results of our rat experiments by a deficiency in the amount or ratio of any group of nutrients, let alone by the lack of a specific trace element such as fluorine, the true significance and mechanism of which — as a caries modifier or as a nutritional essential — remains to be established.

SUMMARY

Evidence has been presented that a purified ration, adequate in known nutrients, is conducive to rat caries independently of the presence of coarse particles and the impact of mastication. Demonstration of a caries producing mechanism operating before tooth eruption, the absence of enamel

fractures and attrition, and the persistence of weakly supported overhanging enamel walls surrounding undermining caries, all tend to indicate that the described lesions are not caused by mechanical injury of the teeth. No difference can be found between the histopathology of the rat caries here presented and presently established findings in man.

ACKNOWLEDGMENTS

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THE TOXICITY OF FLOURS TREATED WITH VARIOUS "IMPROVING" AGENTS[1]

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For more than 25 years dog owners in this country as well as in England have observed in their animals the frequent occurrence of an affliction termed variously canine hysteria, fright fits, or running fits. The etiological agent has been demonstrated by Mellanby ('46) to be flour treated with an "improving" agent[2] known as agene. Agene is defined as 1% NCl_3 in air saturated with water vapor. The purpose of agene treatment is to alter the baking characteristics of freshly milled flour, thereby rendering it immediately usable in modern baking equipment, and eliminating the necessity of prolonged storage. The process has been in increasingly common use for over 25 years. While at the time of Mellanby's report an estimated 80% of the flour used for baking purposes in this country was agene-treated, the use of this agent has now been materially reduced.

[1] Presented before the Division of Agriculture and Food Chemistry at the 112th Meeting of the American Chemical Society on September 18, 1947, in New York, N. Y., and revised by the insertion of references to subsequent findings.

[2] The term "improving agents" is the technical designation in the milling industry for chemicals added to flour for artificial ageing and/or bleaching. In "Definitions and Standards for Food" (Federal Security Agency Service and Regulatory Announcement, Food, Drug, and Cosmetic no. 2) permitted optional ingredients for bleaching and/or ageing are oxides of nitrogen, chlorine, nitrosyl chloride, nitrogen trichloride, and benzoyl peroxide. Potassium bromate not in excess of 75 ppm is an optional ingredient in high protein flours.

Following the publication of Mellanby's original report, papers have appeared in this country and England both confirming his observation and extending the understanding of the phenomenon. Moran ('47) has shown that NCl_3 reacts with gluten, casein, zein and gelatin producing substances which give rise to canine hysteria, and further that the toxic principle cannot be separated from the protein fraction by extraction with organic solvents. These results have been confirmed by both Silver et al. ('47a, b) and Newell et al. ('47) who in addition have reported that monkeys exhibit abnormal encephalograms when fed agene-treated flour. Silver et al. ('47c) have also stated that the amino acids cysteine and cystine when treated with NCl_3 and administered intravenously to dogs produce the same electroencephalographic seizure patterns characteristic of fright fits. A second paper by Mellanby ('47) has shown ferrets to be susceptible to the actions of agenized flour.

The work of this laboratory on the problem was begun soon after the appearance of Mellanby's original report and was directed toward the following objectives: (1) To determine if bread made from agene-treated flour is toxic; (2) to determine whether other treatments commonly used or suggested for use in bleaching or in improving the baking qualities of flour also render it toxic; and (3) to assess the possible hazard of agene-treated flour for human beings.

A brief description of the syndrome termed fright or running fits as produced in dogs by agene-treated flour is in order. The first observable symptoms are general lethargy and muscular incoördination. As the degree of poisoning becomes more advanced, a second stage of the disorder manifests itself in the form of attacks of hysteria in which the animal behaves in a thoroughly panic-stricken manner, throwing itself against the sides of the cage, clawing the air and howling piteously. If unconfined, it will run wildly about bumping into all objects in its path. These attacks may be of only fleeting duration or may last for a number of minutes. The dog usually recovers as suddenly as it is stricken. In very

severe poisoning, a third stage of the disease develops which is characterized by frequent epileptiform or tonic-clonic convulsions. These may become very severe and almost continuous until death occurs. In the experiments to be described, only the occurrence of the second or third stages, that is, actual fits or convulsions, was considered a positive result.

EXPERIMENTAL

Agene-treated flour and bread. Eighteen young mongrel dogs were divided into 4 groups. Group I consisting of 6

TABLE 1

Diets used in dog feeding experiments

	DIET A	DIET B
	gm or ml	*gm or ml*
Flour	150	230
or		
Bread [1]	225	
Milk powder	20	40
Liver	..	10
Horsemeat	15	..
Yeast	7.5	7.5
Salt	1	1
Corn oil	10	20
Baking powder	..	10
Cod liver oil	2.5	2.5
Water	150	150

[1] 225 gm of bread are the equivalent of 150 gm of flour.

dogs was fed flour with 1.4 gm of agene per 100 lb.; group II likewise consisting of 6 dogs was fed untreated flour; group III consisting of 3 dogs was fed bread made by standard baking procedures from the flour used for group I; and group IV also consisting of 3 dogs was fed bread similarly made from untreated flour. The diet used in this experiment was diet A in table 1. The rations containing flour were prepared daily by cooking in live steam for 90 minutes shortly before feeding. The diets containing bread were not cooked. The dogs were fed daily for a period of 6 weeks. They consumed

20 to 40 gm of flour per kilogram per day. The results are shown in table 2. All the dogs in group I fed agene-treated flour and those in group III fed the bread made from this flour developed typical fright fits in a period of feeding ranging from 7 to 17 days. It is clear that standard baking procedure does not diminish the toxicity of agene-treated flour.

Flours treated with agene, chlorine dioxide, chlorine, oxides of nitrogen, benzoyl peroxide and potassium bromate.[3] Groups of 3 dogs each were fed diets containing flours which had been treated with 3 different levels of agene, 2 levels of chlorine dioxide, 2 levels of chlorine and 1 level each of oxides of nitrogen, benzoyl peroxide and potassium bromate. The

TABLE 2

Effects of feeding agene-treated flour and bread

	PRODUCT TESTED	RATIO OF DOGS SHOWING FITS	AVERAGE OF DAYS BEFORE FIRST FIT
I.	Agene-treated flour (1.4 gm/100 lb.)	6/6	15
II.	Untreated flour	0/6	..
III.	Bread made from agene-treated flour	3/3	17
IV.	Bread made from untreated flour	0/3	..

degrees of treatment and the types of flour in each case are shown in table 3. The diet used was diet B in table 1, a slight modification of Mellanby's formula which we found more acceptable to our dogs. The preparation and feeding of the flours were the same as previously described. The daily consumption of flour ranged from 30 to 50 gm/kg/day. The results contained in table 3 demonstrate that of the group of "improving agents" tested only the agene treatment gave rise to fits or any other evidence of toxicity. The levels of treatment as shown in table 3 represent the upper limits of agene treatment commonly used for clear, straight and patent flour, respectively. There is some suggestion that the heavily

[3] These flours were prepared under commercial conditions in the presence of Inspectors of the Food and Drug Administration.

treated clear flour was more toxic than the lightly treated patent flour.

The acute toxicity of agene treated gluten. In an attempt to prepare a product having a consistently high degree of toxicity which would be suitable for acute toxicity studies, commercial gum gluten (80% protein)[4] was treated directly with large quantities of agene. The agene was prepared by

TABLE 3

Effect of feeding dogs with flours treated with various improving agents

AGENT	TYPE OF FLOUR	DEGREE OF TREATMENT	RATIO OF DOGS SHOWING FITS	DAYS BEFORE FIRST FIT
Agene	Clear	3 gm/100 lb.	2/3	20 & 23
	Straight	2 gm/100 lb.	2/3	30 & 35
	Patent	1 gm/100 lb.	1/3	20
Chlorine	Straight	0.5 oz./100 lb.	0/3	..
	Patent	1 oz./100 lb.	0/3	..
Chlorine dioxide	Straight	0.6 gm/100 lb.	0/3	..
	Straight	1.8 gm/100 lb.	0/3	..
Oxides of nitrogen	Straight	11 amperes/100 lb.	0/3	..
Benzoyl peroxide	Straight	1 oz./100 lb.	0/3	..
Potassium bromate	Straight	3.4 gm./100 lb.	0/3	..
Untreated	Straight	.....	0/9	..
	Patent	.....	0/3	..

the method customarily used in commercial practice, i.e., by bubbling chlorine into an aqueous solution of ammonium chloride. The NCl_3 was then aerated from the solution and conducted into a revolving, motor driven box containing the gluten. To obtain a product of maximum toxicity the gluten was treated with increasing amounts of agene until no further appreciable increase in toxicity occurred. With

[4] A product of the Keever Starch Company, Columbus, Ohio.

this heavily treated gluten typical fits and convulsions were obtained when single doses were administered to dogs. Table 4 contains the acute toxicity data which were obtained. Increasing the degree of treatment from 1.5 gm of agene/1000 gm of gluten to 3 gm of agene/1000 gm of gluten increased the toxic effect decidedly. However, doubling the degree of treatment again produced no further significant increase in toxicity. Therefore, saturation was achieved somewhere between the 3 gm/1000 gm and the 6 gm/1000 gm treatment. It must be remembered that the use of the term "saturation" refers only to maximum effect obtained under

TABLE 4

Acute toxicity of agene-treated gluten to dogs

DEGREE OF AGENE TREATMENT	GLUTEN ADMINISTRATION		RATIO OF DOGS SHOWING FITS	MORTALITY RATIO
	Dose	Mode		
gm/kg	*gm/kg*			
1.5	15	Fed	2/3	0/3
3	15	Fed	4/4	3/4
3	5	Stomach tube	1/2 [1]	0/2
3	2	Stomach tube	0/2	0/2
6	5	Stomach tube	2/2 [1]	0/2
6	2	Stomach tube	0/2	0/2

[1] Dogs in both groups showed fits of equally mild intensity.

the conditions of treatment and the particle size of the starting material. It is not meant to imply that further increases in toxicity could not be obtained under different conditions. It is also apparent from these data that the ED 50 (the amount of a substance which produces an effect in 50% of the animals) of this agenized gluten is about 3.5 gm/kg.

Comparative toxicity of other proteins. If, as reported by other investigators, agene reacts with casein and gelatin to yield the same toxic principle which produces fright fits, a comparison of the acute toxicity of these agene-treated proteins would be informative. Highly purified casein and gelatin were treated with the same high level of agene (6 gm/1000 gm) as produced a maximum effect with gluten. These substances

were fed to fasted dogs after mixing with equal quantities of sugar and enough corn oil to make the mixture palatable. The results are contained in table 5. Significantly, agene-treated casein is as potent a fit-producer as agene-treated gluten, while gelatin did not produce fits in the dosage administered. These results obtained with gelatin are not in conflict with those reported by Moran ('47), for this investigator obtained positive results only after a prolonged period of feeding.

In addition, treated gluten was fractionated by extraction with 70% alcohol into its glutenin and gliadin components.

TABLE 5

Acute toxicity of agene-treated proteins to dogs

AGENT	DOSE	RATIO OF DOGS SHOWING FITS	MORTALITY RATIO
	gm/kg		
Gluten	15	4/4	3/4
Casein	15	2/2	2/2
	5	2/2	0/2
Gelatin	100	0/2	0/2
Glutenin	7.5	1/2	0/2
Gliadin	7.5	2/2	1/2

Both these fractions produced fits on feeding but the gliadin appeared to be the more toxic.

Because of the possibility that a simple chemical alteration of a constituent amino acid may be responsible for the toxicity of agene-treated flour, several amino acids in dry form and in solution were treated with agene. These substances were administered by stomach tube to dogs in doses several times larger than the amount of the particular amino acid which would be present in a toxic dose of maximally toxic gluten. No toxic effects were obtained with agene-treated tryptophane, acetyl-tryptophane, tyrosine, methionine, cysteine or cystine. A pancreatic digest of agene-treated gluten was also prepared. Fits and convulsions were obtained when fasted dogs were given 100 ml/kg by stomach tube. (Ten ml con-

tained the products of digestion of 1 gm of gluten.) When the insoluble fraction containing the tyrosine was fed to dogs, no toxicity was obtained.

Variations in species susceptibility. Because toxicity data on a variety of species of animals are of paramount importance in evaluating the possible human hazard, agene-treated gluten was fed or administered by stomach tube to rats, rabbits, monkeys, guinea pigs and cats. The guinea pigs, rabbits and cats received by stomach tube a 25% suspension of 6 gm/1000 gm agene-treated gluten; the monkeys were fed a diet containing 30% agene-treated gluten; the rats were given a diet consisting solely of 1.5 gm/1000 gm agene-treated

TABLE 6

Susceptibility of various species to agene-treated gluten

ANIMAL	DOSE	NO. OF DOSES	MODE OF ADMINISTRATION	NO. OF ANIMALS	RESULTS
	gm/kg				
Dogs	5	1	Stomach tube	4	Fits
Rats	42	30	Fed	6	No fits
Monkeys	15	54	Fed	2	No fits
Guinea pigs	15	1	Stomach tube	10	No fits
Rabbits	15	1	Stomach tube	8	Fits
Cats	15	3	Stomach tube	4	Fits

gluten. The results obtained are shown in table 6. Most noteworthy is the observation that rabbits are of a similar order of susceptibility as dogs. Within 48 hours after the administration of a single dose of 15 gm/kg, rabbits developed fits and convulsions strikingly similar to the syndrome obtained with dogs. Cats developed similar symptoms after 3 administrations. Rats and Rhesus monkeys, however, did not develop any observable central nervous disorders over the prolonged period during which they were fed these large amounts of this exceedingly potent gluten. Guinea pigs did not develop symptoms from a single administration.

The observation of the high degree of susceptibility of rabbits to the effects of agene-treated gluten presents the at-

tractive possibility of their use as an assay animal, not only in testing the potency of various agene-treated proteins, amino acids and other synthetic substances, but also in following the toxicity of fractions obtained during the isolation of the toxic principle from hydrolysates.

DISCUSSION

The question arises as to whether the agene treatment acts by destroying a necessary dietary constituent of flour or by producing a toxic product. The fact in itself that the typical running fit syndrome can be produced in dogs on an acute basis very strongly suggests that the problem is one of simple toxicity uncomplicated by dietary deficiencies.

The question of the identity of the agent responsible for the production of fright fits in dogs remains largely unsettled. That the toxic principle of agene-treated flour is associated with the protein fraction seems to be adequately established. Since proteins are broken down in the animal body and gain access to the blood stream largely as amino acids, it seemed likely that the toxic principle would be a chemically altered amino acid. The report by Silver et al. ('47c) that intravenously administered agene-treated cystine and cysteine will produce characteristic encephalographic seizure patterns in dogs would indicate that the toxic principle was a reaction product of these amino acids and NCl_3. If this were true, the acute oral administration of these agene-treated acids in large doses should have produced a toxic effect. Completely negative results were obtained. However, the possibility still remains that the sulfur-containing amino acids are somehow more complexly involved in the reaction as, for instance, part of a dipeptide. The possibility also remains that an amino acid not yet tested may be responsible. The fact that an enzymatic hydrolysate retained all the toxicity of the agene-treated protein from which it was prepared again suggests an amino acid or a low molecular weight polypeptide. That the toxic principle is a di-, tri- or poly-peptide absorbed intact into the animal's blood stream seems most likely at the present.

Although the data presented definitely demonstrated that the production of fright fits by the agene-treated gluten of flour is not a phenomenon peculiar to the dog but rather a toxic reaction common to several species, the question of possible toxicity to human beings is still unsettled. Obviously a toxicant of such potentialities for harm is not a desirable constituent of the flour from which our daily bread is prepared.

SUMMARY

1. Bread made from agene-treated flour produces fright fits in dogs.

2. Oxides of nitrogen, benzoyl peroxide-, chlorine-, chlorine dioxide- and bromate-treated flours are harmless to dogs when fed for 6 weeks.

3. The ED 50 of gluten "saturation-treated" with NCl_3 is approximately 3.5 gm/kg.

4. The reaction product of NCl_3 and either tyrosine, tryptophane, cystine, cysteine or methionine is not responsible for the production of fright fits.

5. Rabbits are of the same order of sensitivity to agene-treated gluten as dogs and present attractive possibilities for use as assay animals.

6. Cats are also sensitive but less so, while rats and Rhesus monkeys are apparently resistant insofar as the production of gross symptoms is concerned.

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PROLONGATION OF THE LIFE SPAN OF RATS BY BULK-FORMERS IN THE DIET[1]

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McCay ('29, '34) found that rats fed a diet including 10% ground no. 300 glycerine-free cellophane were considerably retarded in growth but lived longer than those fed a stock diet; they also lived longer than rats raised by Campbell ('28) on the superior growth-promoting diet of Sherman and Campbell ('24). The longer life span of the cellophane-fed rats was attributed by McCay ('33, '34) to the retardation in growth rather than to the cellulose in the diet. However, our observations concerning the effect of various types of bulk-formers (Hoelzel and Da Costa, '37; Hoelzel, '39; Hoelzel, Da Costa and Carlson, '41) indicated that ground cellophane was too irritating for liberal use in the diet and suggested that a diet including about 10% of a more suitable type of bulk-former would be likely to prolong the life span of rats without a significant retardation of growth. It was also thought that the roughage in natural foods might serve to prolong life without significantly retarding growth. These possibilities were investigated in connection with our 2 studies of the effects of intermittent fasting and omnivorous and vegetarian diets on the growth and longevity of rats (Carlson and Hoelzel, '46, '47).

[1] This study was aided by a grant from Swift & Company, Chicago.

FIRST LONGEVITY STUDY

Diets used and experimental procedures

Some details concerning our diets and experimental procedures were given in our preceding reports. In our first longevity study, diet 2 (table 1) was used to determine the

TABLE 1

Composition of diets[1]

DIET[2]	
1	Basal or low residue omnivorous diet including 61.5% dried "whole veal,"[3] 3% veal bone meal,[4] 31% cornstarch, 2% dried brewers' yeast, 1.5% inorganic salt mixture and 1% cod liver oil.
2	Diet 1 plus 10% finely ground alfalfa stem meal.
3	Diet 1 plus 5% ground kapoc[5] and 5% psyllium seed husks.[5]
4	Simple vegetarian diet including 50% whole wheat flour, 10% peanut flour, 7% wheat gluten flour, 7% lima bean flour, 7% linseed meal, 7% corn gluten meal, 5% alfalfa leaf meal, 5% brewers' yeast and 2% NaCl.
5	Diet 1 plus 10% semi-fibrous cellulose flour.[6, 7]
6	Vegetarian self-selection diet including corn (whole kernels), wheat (whole grain), pearled barley, rolled oats, sunflower seeds, peanuts, green peas, soy beans, corn germ meal, wheat germ meal, brewers' yeast, alfalfa leaf meal and NaCl.
7	Modification of diet 3 with 5% semi-fibrous cellulose flour[6] in place of 5% ground kapoc.[5]
8	Diet 1 plus 10% granular (80 mesh) karaya gum[7] gradually increased to 25% and granular gum acacia[7] used in place of karaya gum during 2 months.
9	Modification of diet 7 — diet 1 plus 6.7% semi-fibrous cellulose flour and 3.3% psyllium seed husks.[5]
10	Diet 1 plus 10% ground (40 mesh) glycerine free no. 150 cellophane.[7]
11	Diet 1 plus 20% semi-fibrous cellulose flour and 5% psyllium seed husks.
B	Refers generically to diets with 10% added bulk (diets 3, 5, 7, 9 and 10).

[1] Trimmings of head lettuce were fed ad libitum as a supplement to all diets.

[2] Changes in diet are indicated by hyphenated figures. Thus, diet 3–7–9 indicates a change from diet 3 to diet 7 and finally to diet 9. In general, only 1 change was made, such as in diets 1-B, B-1 and 5-10.

[3] Especially prepared for us by Swift & Company, Chicago.

[4] Obtained from Mead Johnson & Company's laboratory.

[5] Freed from seeds, boiled, washed, partly bleached and dried.

[6] Cellu Flour B.

[7] Obtained from The Chicago Dietetic Supply House.

effect of a natural source of roughage and an amount of roughage, added to our basal omnivorous diet (diet 1), that served to produce approximately the same volume of feces as our simple vegetarian diet (diet 4). Diet 3 was used to determine the effect of a type of bulk-former regarded as more satisfactory than the ground cellophane used by McCay (Hoelzel, '39) and fed in an amount approximately equal to 10% cellophane. In this study, 45 rats were fed diet 1, and 2 groups of 23 each were given diets 2 and 3, respectively. Besides this, separate life span observations were made on 6 second generation rats on diet 2. Approximately equal numbers of the rats were fed the diets ad libitum or fasted 1 day in 4, 3, or 2.

Results

The rats fed diet 2 did not gain weight as rapidly as the animals on diet 1, but they gained weight more rapidly than those fed diet 3. The maximum weights attained, femoral lengths and ages at death of the rats are presented in table 2, but not the specific effects of the different degrees of fasting. Although the rats fed diets 2 and 3 did not become as heavy as the animals fed diet 1, they attained the same size (femoral length). The rats on diet 2, including the 6 second generation rats on this diet, did not live longer than those on diet 1, but the animals on diet 3 lived longer (average, 77 days longer). Most of the fasted rats on diets 2 and 3 lived longer than those fed ad libitum, but the precise effect of fasting could not be clearly evaluated from the limited amount of data.

A search for lower bowel lesions revealed no gross evidence of chronic or ulcerative cecitis but diverticula of the colon near the cecum, such as Wierda ('43) discovered, were found, particularly in rats on diets 1 and 2. Details will be reported in a separate paper.

The females on diets 2 and 3 did not develop as large mammary tumors as those on diet 1 but there was no significant difference in the proportion of females developing this condition.

TABLE 2

Maximum weights, femoral lengths, and ages at death of rats fed a low residue omnivorous diet (diet 1), this diet with added bulk-formers (diets 2, 3, 5, 7, 8, 9, 10 and 11) and a vegetarian self-selection diet (diet 6), ad libitum or with intermittent fasting during 2 longevity studies

DIET [1]	NO. OF RATS		MAXIMUM WEIGHT [2]		FEMORAL LENGTH [2]		AGE AT DEATH [3]	
	♂	♀	♂	♀	♂	♀	♂	♀
			gm		*mm*		*days*	
FIRST LONGEVITY STUDY								
Rats fed ad libitum								
1	5	7	591	456	39.0	34.7	633 ± 123	659 ± 125
2	2	3	566	389	40.3	35.0	552 ± 147	785 ± 102
3	2	3	466	453	39.4	35.2	661 ± 145	742 ± 19
Rats fasted 1 day in 4, 3 or 2								
1	14	19	407	325	38.1	34.8	667 ± 143	758 ± 114
2	7	11	383	285	38.3	34.6	637 ± 113	725 ± 200
3	9	9	399	292	38.5	35.3	738 ± 191	842 ± 201
SECOND LONGEVITY STUDY								
Rats fed ad libitum								
1	4	3	639	551	39.8	33.2	588 ± 96	701 ± 129
3–7–9	2	3	592	441	38.3	34.6	718 ± 38	751 ± 122
5	5	4	668	421	37.9	35.3	807 ± 191	646 ± 61
10	1	1	674	336	38.6	32.9	842	418
5–10	2	2	758	397	38.7	34.2	691 ± 5	692 ± 141
1–B	3	2	649	453	39.1	33.3	864 ± 175	911 ± 127
B–1	4	4	658	448	37.8	34.3	687 ± 57	850 ± 211
1–11	2	1	506	316	38.3	34.4	906 ± 158	937
B–11	5	5	497	398	37.4	33.8	692 ± 243	937 ± 72
8	2	1	650	410	38.2	34.4	920 ± 11	779
1–6	1	1	524	362	40.9	34.3	564	585
B–6	1	2	576	422	35.9	34.4	718	853 ± 119
6–1	1	1	570	244	37.8	32.1	511	379
6–B	3	3	572	383	38.2	34.8	845 ± 262	826 ± 95
Rats fasted 1 day in 3								
1	3	2	537	355	38.0	33.3	676 ± 136	930 ± 26
3–7–9	2	2	502	427	38.5	34.9	630 ± 220	840 ± 44
5	3	4	531	398	37.7	34.6	730 ± 183	972 ± 124
10	1	1	512	430	37.7	35.9	845	777
5–10	1	2	550	366	38.2	34.4	863	983 ± 105
1–B	1	2	470	402	38.0	34.0	636	808 ± 95
B–1	2	4	495	393	37.8	34.0	715 ± 132	935 ± 25
8	1	2	450	341	36.8	33.3	708	867 ± 20
1–6	1	1	458	330	38.3	32.9	470	835
B–6	2	1	446	282	37.6	33.9	550 ± 73	645
6–1	0	0						
6–B	1	2	436	262	37.7	32.9	798	799 ± 383

[1] See table 1, footnote 2, concerning changes in diets.
[2] Mean.
[3] Mean and standard deviation.

Discussion

The failure of the rats on diet 2 to live longer than those on diet 1 in spite of their lower maximum weights was apparently partly due to the unsatisfactory nature of alfalfa stem meal as a source of roughage. It seemed that it introduced some deleterious substance(s) which increased the incidence and severity of respiratory infections. Alfalfa stem meal also did not seem to provide enough hemicellulose to keep the feces from becoming too firm for easy passage. Wheat bran might have served as a better natural source of roughage, but alfalfa stem meal was used in preference to wheat bran because it introduced less vegetable protein into the diet which was intended to contain mainly meat protein. However, even if a more satisfactory source of roughage than alfalfa stem meal had been used, the amount of roughage provided would not have been likely to increase the life span significantly. This is suggested by the results obtained with diet 3 which provided at least twice as much of a more satisfactory type of bulk and apparently introduced nothing of a deleterious nature. Although the rats on diet 3 lived, on the average, 77 days longer than those on diet 1, more data seemed needed to prove that this extension of the life span was statistically significant. A second study was therefore begun in which the main object was to secure more data on the effect of various kinds and amounts of relatively pure bulk-formers on growth and longevity.

SECOND LONGEVITY STUDY

Diets used and experimental procedures

Some details concerning the diets and experimental procedures in our second longevity study were also given in a preceding report ('47). In this study, diets 3, 5, 7, 8, 9, 10 and 11 (table 1) were used to secure data on the effect of bulk-formers added to diet 1. Diets 3, 5, 7, 9 and 10 were alike in involving the addition of 10% bulk to diet 1 and these diets are therefore generically referred to in some connections as diet B. Diet 3 was replaced by diet 7 because the supply of kapoc

was cut off by the war and, later, diet 7 was replaced by diet 9 to conserve the supply of psyllium seed husks. Rats fed these diets are therefore referred to as rats on diet 3-7-9. Diet 5 served to determine the effect of the cellulose flour used in diets 7 and 9 without added hemicellulose in the form of psyllium seed husks. Diet 10 served to determine the effect of a form of cellulose less irritating than the ground no. 300 cellophane used by McCay: the no. 150 cellophane used here was only half as thick as no. 300 cellophane. Diet 11, with 25% added relatively smooth bulk, was used to compare its effect with McCay's ('34) use of a diet including 20% ground no. 300 cellophane. Diet 8 was used mainly to determine whether karaya gum would produce ulcerative cecitis in the (Wistar) rats that we were using as they did not develop ulcerative cecitis "spontaneously" like the rats that we had used in a previous test of the effect of karaya gum (Hoelzel, Da Costa and Carlson, '41). Gum acacia was used in place of karaya gum in diet 8 during a period in which suitable karaya gum was not obtainable.

Eighteen litters, including 115 rats, were used in this study. The original intention was to use a much larger number of rats but wartime and other conditions beyond our immediate control prevented this. Five of the 18 litters were started on diet 1, 4 on diet 3-7-9, 6 on diet 5, 1 on diet 10 and 2 on the vegetarian diet 6. However, 2 of the 6 litters started on diet 5 were so started merely in preparation for being placed on diet 10 when about 100 days old. Fourteen rats started on diet 1 and 16 started on diet B (diets 3-7-9, 5, 10 and 5-10) were kept on these diets throughout life. Nine rats started on diet 1 were placed on diet B when 72 to 200 days old. Six rats (1 male and 1 female from each of 3 litters started on diets 1, 5 and 6) were placed on diet 8 when 42 days old. Eight males and 7 females started on diets 1 and B were placed on diet 11 when 147 to 200 days old. All rats started on the vegetarian diet 6 were placed on diet 1 or diet B before they became 345 days old and 10 rats started on diets 1 and B were placed on the vegetarian diet when 72 to 200 days old. All rats on diet

11 were fed ad libitum throughout life. About half (45) of the other 100 rats in this study were fasted 1 day in 3 after they became 100 to 200 days old.

The results were statistically evaluated by calculating *t* values according to the small sample methods explained by Fisher ('37) and Snedecor ('46) and probability (P) values of less than 5% were regarded as indicating significant differences.

Results

Growth curves showing the weights between the ages of 42 and 200 days of the rats fed diets 1, B and 6 ad libitum were included in our preceding report ('47). The maximum weights attained, femoral lengths, and ages at death are presented in table 2. However, data on 5 rats that died relatively early and apparently because of factors other than diet or fasting are not included in table 2. One of the 5 excluded rats was an intermittently fasted male on diet 5-10. This animal died of a rapidly growing tumor on the nape of the neck when 358 days old. The other 4 excluded rats were 2 males and 2 females that constituted all of the young in the last (7th) litter of one of our breeding females that were reared. This litter was started on diet 1 and kept on this diet until 147 days old. After that, 1 male and 1 female were intermittently fasted and the other male and female were placed on diet 11. The males died when 329 and 345 days old and the females when 477 and 522 days old, respectively. In each case, the rat fed diet 11 lived longer than the fasted litter mate kept on diet 1. The rats raised in the last litters of our 2 other breeding females that were mated with the same male lived to be 635 to 1018 days old (6 males) and 741 to 937 days old (3 females), respectively. One of these litters, including 4 males and 3 females that were reared, also had been started on diet 1. A male of this litter placed on diet 11 when 150 days old lived to be 1018 days old, and a female on the same regimen became 937 days old. The exclusion of data on the 4 rats in the short-lived last litter therefore seems amply justified.

The data in table 2 indicate that the average weight attained by the males fed diet B ad libitum was greater than that of the males fed diet 1 ad libitum in spite of the fact that growth was slower on diet B. The females on diet B, however, did not become as heavy as those on diet 1. This sex difference in attained weights seems largely explainable by the effects of the diets on the life spans. The males on diet B lived longer than those on diet 1 (average, 181 days longer — $P<5\%$). Obviously, the longer life span of the males on diet B included a longer period during which growth continued. The females on the other hand, particularly those on diets 5, 10, and 5–10, did not live longer than those on diet 1 and therefore did not attain similar weights. The skeletal growth (femoral length) of the males started early on diet B appears to have been stunted slightly; this was not true of the females. Both males and females fed diet 1 early in life and diet B later in life (diet 1–B) lived longer than those fed either diet ad libitum throughout life. The data on the reversed regimen (diet B–1) only indicate that the opposite regimen is superior. The rats fed diet 11 after diet 1 or B (diet 1–11 or B–11) lived longer than those kept on diet 1 or B. This is not indicated by the data in table 2 concerning the males on diet B–11, but 4 of the 5 males had litter mates that were kept on diet B and all of the litter mates fed diet 11 lived longer (average, 83 days longer). The females on diet B–11 lived much longer than those kept on diet B (average, 273 days longer — $P<1\%$) but this difference is partly explainable by the relatively short life spans (average, 664 days) of the females kept on diet B.

The life spans of the rats fed diet 8 were similar to those of rats fed other bulk-formers but the karaya gum appeared to have a depressive effect on the rats. This seemed to be partly due to a reduction of the food intake because of the unpalatability of the gum. It was originally intended to use only 10% (added) karaya gum, but the volume of feces produced was very small. Even 25% karaya gum did not produce very voluminous feces, presumably because of the relatively

low food intake and a breakdown of the gum in the digestive tract. The food intake increased and the weights increased rapidly when the more acceptable gum acacia was used. The alertness of the rats also increased then but the volume of feces decreased because of an even greater breakdown of gum acacia in the digestive tract.

TABLE 3

Summary of effects of diets fed ad libitum on life span of rats in second longevity study

DIETS	MALES		FEMALES		SUITABILITY OF DIET
	No.	Age attained [1]	No.	Age attained [1]	
Omnivorous					
3–7–9, 1–B, 1–11, B–11, 8	14	795 ± 192	12	873 ± 116	Relatively satisfactory for both sexes
1, B–1	8	638 ± 90	7	786 ± 196	Less satisfactory for males than females
5, 10, 5–10	8	782 ± 156	7	626 ± 119	Less satisfactory for females than males
Part-time vegetarian					
B–6, 6–B	4	813 ± 223	5	836 ± 91	More satisfactory for both sexes than diets 1–6 and 6–1
1–6, 6–1	2	538 ± 38	2	482 ± 146	Less satisfactory for both sexes than diets B–6 and 6–B

[1] Mean and standard deviation.

Tables 2 and 3 show that the rats fed diet B before or after the vegetarian self-selection diet (diets B–6 and 6–B) lived longer than those fed diet 1 before or after the vegetarian diet (diets 1–6 and 6–1).

The intermittently fasted males on diet B did not live longer than those fed diet B ad libitum. The intermittently fasted females on diet B lived longer (average, 259 days longer — $P<1\%$) than those fed diet B ad libitum, but this great

difference is again largely due to the relatively short life spans of the females fed diet B ad libitum.

No grossly evident cecal lesions were found in the rats on diet 8 but further evidence was found that the development of diverticula of the colon was promoted more by the low residue diet than by the bulky diets. Differences in the effects of the different bulk-formers were nevertheless also noted and the details will be reported in a separate paper.

The incidence of mammary tumors did not seem to be reduced by the bulky diets. In this study, also, the size attained by the tumors was not clearly reduced by diet B (10% added bulk) but it was definitely reduced by diet 11 (25% added bulk).

Discussion

A further study will be needed to explain the differences in the effects of the different bulk-formers on the 2 sexes and under different conditions. The differences in rats fed ad libitum are clearly indicated in table 3. Comparisons between litter mate male and female rats on diet B showed that the males fed ad libitum lived 174 days longer than the females fed ad libitum but that the intermittently fasted females lived 158 days longer than the fasted males ($P<5\%$ in both comparisons, which involved 8 and 7 pairs of rats, respectively). The complicated nature of the sex difference is further emphasized by the fact that the 5 females on diet B–11 (without fasting) lived as long as their intermittently fasted litter mate females on diet B. This was also true of 5 additional pairs of females, of which 1 of each pair was started or continued on diet 1 and the other was placed on diet 11, and 10 pairs of litter mate males started on diets 1 and B. This incidentally indicates that diet 11 (sufficient bulk but including psyllium seed husks) fed ad libitum served as well as fasting 1 day in 3 in prolonging the life spans.

The types and amounts of bulk-formers that would serve best at different ages to promote the greatest length of life without stunting growth or impairing vigor also need further clarification. Our results suggest that a suitable type and

proportion of hemicellulose as well as cellulose should be included in the diet and that no bulk-former or only small and gradually increasing amounts should be used at early ages. Psyllium seed husks seem to provide a suitable type of hemicellulose. We did not determine the effect of using psyllium seed husks alone because the mucilage which the husks alone form cannot be segmented normally in the digestive tract. The mixtures of kapoc or cellulose flour and psyllium seed husks which we used were apparently segmented normally but the proportions that might serve best remain unsettled. Our observations suggest that as much as 10% bulk should not be used before rats are about 150 days old. Diet 11, with 25% added bulk, was evidently also too bulky for some rats 200 days old, but it seemed to be satisfactory for the rats when they were over 500 days old. This may have been partly due to the naturally reduced food intake with ageing. The life span of some of our bulk-fed rats may therefore have been prolonged partly by a slowing down of living processes during some periods by the diets being too bulky to permit a sufficient caloric intake, but we believe that the life span of most of our bulk-fed rats was prolonged without any slowing down of living processes. During middle life, some of the rats on diet B were unquestionably more active or alert than any rats on diet 1. This may, of course, merely mean that the activity of the rats on the low residue diet was depressed. On the other hand, the prolongation of the life span of the rats fed the bulky diets ad libitum was evidently not due to increased activity such as often occurs on the days of fasting in intermittently fasted rats.

The food intake of our rats was not determined, but large amounts of non-nutritive materials in the diet obviously reduce the caloric intake by filling the digestive tract and dispelling the desire to eat with the ingestion of less nutriment (Hoelzel, '47). Theoretically, the food intake with ad libitum feeding can be reduced to any desired level by simply including the necessary proportion of inert material in the diet, but the optimum amount of a bulk-former that can be included

in the diet depends on the nature of the bulk-former and the nutritional requirements for normal growth and the maintenance of vigor. It is evidently more practical to prevent the ingestion of excessive amounts of nutriment by feeding a bulky diet ad libitum than by intermittent fasting or by feeding restricted amounts of a relatively low residue diet daily. The possibility also exists that suitable bulk-formers help to prolong life by normalizing intestinal conditions. This is suggested partly by the finding that colonic diverticula were absent in practically all rats fed the diets including psyllium seed husks.

Our second life span study was designed partly to secure data concerning the relative influence of heredity and litter order as well as nutrition on the life spans but much more data than we secured would be needed for such a purpose. Nevertheless, the relatively early death of all of the rats in one of the 3 last litters suggests that last litters or offspring of ageing rats are not likely to live as long as offspring of younger rats. First litters were not used in our second study but the data obtained on second to last (6th, 7th and 8th) litters indicated that the constitutions or potential life spans of the rats in litters after the 4th were at least more frequently poor than among the rats in the earlier litters. Data on successive generations, such as Lansing ('47) obtained on rotifers, would evidently be necessary to determine the specific effect of ageing on the life span of offspring.

The failure of cecal ulceration to develop in the rats fed karaya gum in this study suggests that this gum merely increased "spontaneous" cecal ulceration in the rats used in our previous studies (Hoelzel, Da Costa and Carlson, '41, and unpublished observations). In short, the view of Bloomfield and Lew ('43) that ulcerative cecitis in rats is of infectious origin is indirectly supported. However, the fact remains that karaya gum and apparently also some other bulk-formers increase ulcerative cecitis in infected rats.

SUMMARY

The effects of feeding a low residue diet and this diet with added bulk-formers on the growth and longevity of 212 Wistar rats were determined. The bulk-formers used included alfalfa stem meal, psyllium seed husks, ground kapoc, cellulose flour, ground cellophane and granular karaya gum. The effects of feeding the different diets ad libitum or with intermittent fasting, and of feeding the low residue or bulky diets early or late in life were simultaneously investigated.

The results indicated a sex difference in the effect of bulk-formers. In rats fed ad libitum the life span of both sexes was increased by 10% and 25% (added) relatively smooth bulk-formers but the life span of females was not increased by 10% cellulose flour or ground cellophane. The results differed in intermittently fasted rats and in those fed different amounts of bulk during different periods of life. No impairment of skeletal growth was produced when diets with 10% bulk-formers were fed only after the rats became 42 or more days old. A diet with 25% relatively smooth bulk prolonged the life span as much as fasting 1 day in 3, which was previously found to be the optimum amount of intermittent fasting for rats.

Contrary to findings in previous studies, no cecal ulceration was found in 6 rats fed 10% to 25% granular karaya gum.

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THE TOTAL SPECIFIC DYNAMIC ACTION OF HIGH-PROTEIN AND HIGH-CARBOHYDRATE DIETS ON HUMAN SUBJECTS[1]

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TWO FIGURES

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The estimation of the fuel value of human diets by the application of the Atwater factors of 4, 4 and 9 cal. per gram of total protein, carbohydrate and fat, respectively, allows for average losses of food energy in digestion, and the incomplete oxidation of protein in metabolism under conditions of nitrogen equilibrium. It does not, however, take account of the stimulating effect of the food on metabolism, the specific dynamic action. The energy thus dissipated, except under environmental conditions otherwise tending to induce a negative heat balance, is a wastage of food energy as far as its value in promoting physiological work is concerned. Allowance must, therefore, be made for this wastage of energy in computing the amount of food energy required for persons of given size, age and activity. Hence, the amount of this wastage is of importance in practical dietetics.

[1] The data presented in this report were secured in an experimental project covered by a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Illinois.

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For this purpose, the specific dynamic action (S.D.A.) of food should be measured as the total extra heat produced post prandium above a baseline level. Of the many investigations that have been reported on the specific dynamic effect of food in humans, relatively few involve the measurement of the extra calories above basal produced by a meal of definite description, and very few indeed extend over a sufficiently long period of time post prandium to measure the total effect. The most complete study of this nature was reported by Benedict and Carpenter ('18), involving many experiments and many subjects. The general conclusion from this monumental work was that the specific dynamic action of pure carbohydrates and predominantly carbohydrate meals amounted to about 6% of the fuel value of the food ingested; for fats, the average value is about 2%, while with a protein-rich diet, the S.D.A. approximates 12% of the metabolizable energy (fuel value) ingested. For a mixed diet, "which more properly corresponds with every-day usage," a value of 6% is deduced from the experimental data. Concerning these results, the authors say ". . . in drawing conclusions from the results given in these 2 tables, it should be remembered that the figures given are low, rather than maximum values, since in the majority of instances the basal value was not reached before the conclusion of the experiment . . ." (p. 343).

In observational periods extending over 6 to 10.5 hours post prandium, Murlin, Burton and Barrows ('36) report an average S.D.A. of fat in human subjects of 4.74% of the caloric content. For periods up to 7 hours post prandium, Krauss and Rettig ('29) secured an average S.D.A. for protein foods of about 32%, but neither the experiments of Abel ('43) nor those of Jahn and Strössenreuther ('28) indicate that the S.D.A. of proteins, or of high-protein foods or meals, terminates within a period of 6 or 8 hours. The common practice of deferring a determination of the basal metabolism of men and women until 12 or more hours have elapsed since the last meal, with the stipulation that the last meal should not be high in protein, carries the same implication.

Wachholder and Franz ('44) studied the S.D.A. of a mixed diet in a group of about 50 normal men; observations were made intermittently throughout the day, during which the usual 3 meals were served. The interval between breakfast and lunch was 5 hours, and that between lunch and supper, 5.5 hours. The total observed S.D.A. for the breakfasts, varying in size from 536 to 922 cal., averaged 6.6% of the calories consumed; that for the lunches, containing 812 to 1350 cal., averaged 9.5% of the consumed calories. It is not probable that the latter value includes all of the extra heat produced after lunch, but nevertheless the results show a distinct tendency for a greater proportion of the food calories to be dissipated as S.D.A., the larger the caloric content of the meal.

Post-prandial metabolism in the human is ordinarily observed by intermittent determinations of heat production following a meal of a definite description. During the period of observation, the subject must be inactive. To continue the observations until the S.D.A. of the experimental meal runs out may be extremely tiresome for the subject, and if accomplished may give metabolic measurements complicated by appreciable and variable activity. Wachholder and Franz ('44) took particular pains in their protracted experiments to make their subjects comfortable and to obviate this criticism; with what success we do not know. The purpose of the report to be made below is to present experimental data on the specific dynamic action of high-protein and high-carbohydrate meals in 2 groups of 6 adult men, and in particular to present a method of summating the extra calories post prandium over a reasonably long observational period of 6 to 7 hours and of estimating by extrapolation the total extra calories that would have been measured if the observations had extended over the entire period of elevated metabolism.

EXPERIMENTAL PROCEDURE

In connection with a comprehensive project involving the study of the effect of dietary modifications on the tolerance

of man to repeated exposure to intense cold, a series of tests were made on the 12 subjects of the experiment to measure the specific dynamic action (the heat increment) of the high-protein and the high-carbohydrate diets used in the first year's work. These tests are referred to by Keeton and associates ('46). The following quotation from this publication briefly describes the S.D.A. experiments:

"The heat increment of the experimental diets was determined by following the heat production of the subjects in the respective diet groups for a period of six hours after consumption of the 'A' meal containing approximately 1000 calories. The experiments were carried out with the subjects sitting quietly in a chair, under comfortable conditions (77°F.), and again under cool conditions (60°F.). In both series of tests the men were clothed only in light union suits, 90% cotton. The respired air was collected intermittently, every half hour for the first 2 hours post prandium, and then every hour."

For each S.D.A. measurement, the fasting metabolism of the subject, at least 12 hours after the last meal, was determined while sitting quietly in a chair. The heat production in all tests was estimated from the oxygen consumption and the total respiratory quotient, neglecting protein metabolism. The error made by this neglect is usually less than + 2% (Mitchell and Haines, '27), and under extreme conditions would not exceed + 6.58%. It is well known that the computation of non-protein respiratory quotients, for short periods of time and during variable rates of protein metabolism, on the basis of the nitrogen excretion in the urine, cannot be done with any degree of accuracy.

There were 12 subjects, men varying in age from 23 to 25 years. The subjects are described individually and in detail by Keeton et al. ('46, table 1). Their average weight was 69.7 kg and their average body surface area was 1.865 m^2 during the time the S.D.A. tests were carried out.

EXPERIMENTAL RESULTS

A description of the average experimental meals is given in table 1. These values were calculated from the amounts of the 2 diets consumed and the average composition of such foods taken from Bowes and Church ('42), supplemented by such direct analyses as were necessary. As regards the total day's food, these estimates were checked occasionally by the chemical analysis of composite samples of food, each taken

TABLE 1

Description of average test meals

DIET	ENERGY VALUE		DISTRIBUTION OF CALORIES		
	Total	Per m^2 body surface	Protein	Fat	Carbohydrates
	cal.	*cal.*	%	%	%
High-protein	993	532	37	46	17
High-carbohydrate	1070	574	7	39	54

TABLE 2

Typical test meals for 2 diet groups

HIGH-PROTEIN MEAL		HIGH-CARBOHYDRATE MEAL	
	gm		*gm*
Tomato juice	250	Grapefruit juice	180
Beef	200	Puffed wheat	12
Cottage cheese	125	Cream	50
Milk	50	Bread	60
Bread	20	Milk	325
Butter	15	Butter	25
2 eggs		Sugar	23
1 egg white		Jam	50

over a 10-day period. The test meals were not all the same, but their calculated contents of proteins, fats and carbohydrates were fairly well equalized. The mineral and vitamin contents of the day's food were shown to be adequate by direct analysis.

Typical test meals of the 2 diet groups are shown in table 2.

The test meals in both diet groups averaged about 1000 cal. in fuel value, with ranges from 826 to 1136 cal. for the high-

protein group, and from 787 to 1325 cal. for the high-carbohydrate group. There was no observable correlation between the caloric intakes and the cumulative S.D.A. over a period of 6.5 hours post prandium. This was also the experience of Murlin, Burton and Barrows ('36) in their studies with high-fat diets. Apparently other factors determining heat production, not under experimental control, obscured the correlation that undoubtedly exists between the caloric content of meals of similar character and their calorigenic response in the body.

The data for the individual metabolism tests are too numerous to present in this paper. They were averaged for the 2 diets and the 2 environments for the intervals 0.5, 1.0, 1.5, 2.0, 2.5, 3.5, 4.5, 5.5 and 6.5 hours post prandium. All tests

TABLE 3

The average fasting sitting metabolism of the 2-diet groups of six men each

ENVIRONMENT	HIGH-PROTEIN DIET		HIGH-CARBOHYDRATE DIET	
	Cal./hr./m²	R.Q.	Cal./hr./m²	R.Q.
Comfortable	35.3	0.760	35.3	0.793
Cool	35.1	0.757	37.8	0.776

included in these averages were carried out within 15 minutes of the times indicated. In thus pooling the data for the different subjects, total heat productions for the time interval since conclusion of the experimental meal were calculated and the heat increments computed by deducting the fasting metabolism for the same time interval. A few of the data secured could not well be included in the group averages, either because the calorie content of the test meal was too small or because the time of testing was more than 15 minutes from the times selected for grouping.

The average fasting sitting metabolic rates of the 2 groups of 6 men each in the 2 environments are summarized in table 3. The temperature of the room did not have an appreciable effect upon the metabolic rate of the men on the high-protein diet. For the other diet group, the average metabolic rate

was 37.8 cal. per m^2 in the cool room and 35.3 cal. per m^2 in the comfortable room. An analysis of the differences in metabolic rate for each of the 6 men when in the cool room and in the comfortable room revealed 2 exceptions for which the rate was higher in the comfortable room. For the entire group of 6 men the probability is 0.12 (Student, '25) that the average group difference might have been produced by a random combination of the uncontrolled factors in the experiment.

The average elevation of the fasting metabolism throughout the 6.5 hours of observation was somewhat higher for the subjects of both diet groups in the cool room than in the warm room, but the difference was neither great nor of statistical significance. For the high-protein group, the cumulative S.D.A. for 6.5 hrs. averaged only 2.3 cal. higher for the tests in the cool room, a 1.9% increase. For the high-carbohydrate group, the average cumulative elevation was 4.3 cal., a 5.3% increase. However, this increase was not exhibited by 2 of the 6 subjects in this group.

The irregularities in the data from subject to subject and the inappreciable effect of the experimental environments on the results secured, if any effect at all was manifested, justified pooling the measurements secured in the 2 rooms for each of the experimental diets. The average values obtained in this way are summarized in table 4.

For the high-protein meals, the cumulative S.D.A. over the period of observation averaged 131.1 cal.; for the high-carbohydrate meals, the average was 85.3 cal. The calorigenic response of the high-protein meals exceeded that of the high-carbohydrate meals from 1.5 hours post prandium to the termination of the tests. On the former meals, the respiratory quotients exhibited a slight rise in the first 2 hours to a high of 0.793, and then decreased continuously to 0.760. On the high-carbohydrate meals, the R.Q. rose to 0.902 in 0.5 hr. and then decreased rather regularly also to 0.760, when the measurements were discontinued.

The purpose of the statistical analysis of the above data is twofold: (a) to smooth out the raw data by fitting to them a

TABLE 4

The average results of the metabolism trials

HOURS POST PRANDIUM	HIGH-PROTEIN DIET					HIGH-CARBOHYDRATE DIET				
	Number of tests	Number of subjects	Average R.Q.	Cumulative S.D.A.[1]		Number of tests	Number of subjects	Average R.Q.	Cumulative S.D.A.[1]	
				Observed cal.	Calculated [2] cal.				Observed cal.	Calculated [3] cal.
0	14	6	0.757	0	....	18	6	0.776	0	...
0.5	2	2	0.804	10.2	....	5	5	0.902	12.0	...
1.0	17	6	0.784	19.1	....	17	6	0.880	19.1	...
1.5	18	6	0.793	32.0	....	18	6	0.864	28.4	28.2
2.0	17	6	0.791	44.4	41.3	17	6	0.850	38.2	38.1
2.5	18	6	0.782	57.1	56.8	14	5	0.837	46.4	46.7
3.5	18	6	0.776	80.9	82.5	14	5	0.817	61.5	60.9
4.5	18	6	0.770	102.4	102.5	14	5	0.785	70.9	71.8
5.5	14	6	0.770	118.7	118.1	12	6	0.795	81.0	80.2
6.5	13	6	0.760	131.1	130.3	11	5	0.760	85.3	86.7

[1] Specific dynamic action expressed in cumulative extra calories above basal heat production.

[2] Predicted from equation 1.

[3] Predicted from equation 2.

suitable mathematical equation, permitting interpolation for any time post prandium, and, by differentiation, estimates of rates of extra calorie production at any time; and (b) to arrive at some reasonable estimate of the total cumulative S.D.A. for each type of meal, approximating what would have been obtained had the observations been continued until the fasting sitting metabolic rate had again been observed. On plotting the cumulative S.D.A.'s given in table 4 against hours post prandium, a pattern of points was obtained exhibiting a sigmoid shape, similar to a growth curve, with the self-accelerating phase confined to the first 1.5 to 2.0 hours post prandium. The data collected beyond this time seemed to be well described by the equation for the law of diminishing returns, as used by Brody ('45, p. 502) in his growth studies. Fitting the equation to the data of the 2 diet groups by the graphic method of determining the constant A (see Brody, p. 512) and by the method of averages in determining the constants B and k, the following equations were obtained:

for the high-protein group

$$S_p = 173 - 217.3^{-0.29291t} \qquad (1)$$

and for the high-carbohydrate group

$$S_c = 108 - 118.5^{-0.26176t} \qquad (2)$$

in which S_p and S_c are the cumulative S.D.A.'s for the high-protein and high-carbohydrate meals, respectively, expressed in calories, at time t, expressed in hours post prandium, and e is the base of the natural system of logarithms.

In figure 1, the experimental observations are plotted together with the above equations. The fit of the equations to the respective sets of data is extremely good at 1.5 hours post prandium and beyond for the high-carbohydrate diet, and at about 2.0 hours and beyond for the high-protein diet (see table 4). The course of the data prior to these times is of the self-accelerating, rather than of the self-inhibiting, type as these qualifying terms are used by Brody.

In equations (1) and (2), the first constant terms on the right side of the equations, i.e., 173 and 108 cal., are the

asymptotes to their respective curves, the values the curves are approaching but will never reach in finite time. They are, therefore, somewhat higher than the total S.D.A.'s.

In applying these equations to mammalian growth, the constants signify the mature weights of the respective species at infinity. For practical purposes, Brody considers the

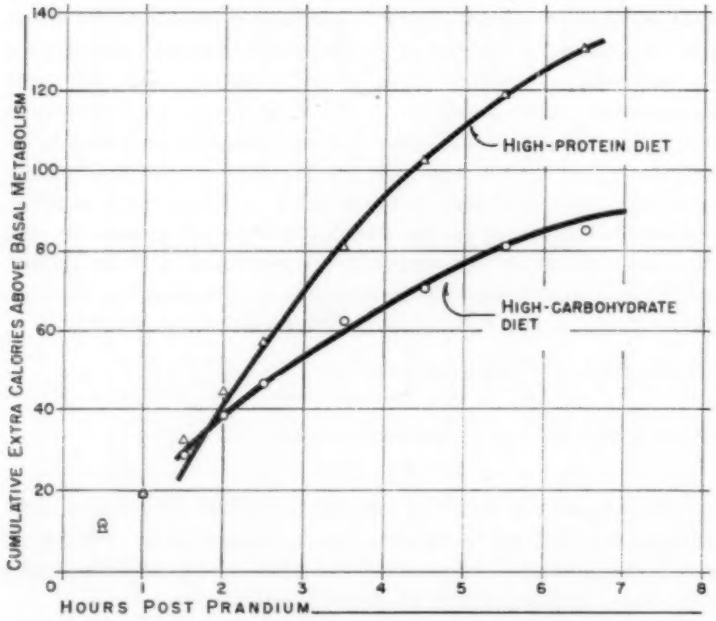

Fig. 1 The increase in cumulative extra calories (S.D.A.) with time post prandium for high-protein and high-carbohydrate meals.

mature weight to equal 98% of the A constant. Applying the same conception to the S.D.A. equations, the total S.D.A. for the high-protein diet would be estimated at 170 cal. (0.98×173), a value that would be attained in 16.54 hours post prandium. For the high-carbohydrate diet, the estimate for total S.D.A. (108×0.98) is 106 cal. and would be attained in 15.12 hours post prandium. At the end of 12 hours post

prandium, the time ordinarily assumed as the length of the absorptive period in the human provided the last meal is not too high in protein, the cumulative S.D.A. for the high-protein diet is 162 cal., and for the high-carbohydrate diet, the value is 102 cal.

By differentiating equations (1) and (2), derived equations are obtained from which may be estimated the instantaneous rate of extra heat production at any time during the absorptive period. The differential equations are:

$$\frac{dS_p}{dt} = 54.35^{-0.256t} \qquad (3)$$

for the high-protein diet, and

$$\frac{dS_c}{dt} = 31.25^{-0.263t} \qquad (4)$$

for the high-carbohydrate diet. These equations are plotted in figure 2, starting with the time post prandium at which equations (1) and (2) describe the experimental data. The differentials $\frac{dS}{dt}$ will evidently be expressed in extra calories above the fasting sitting metabolism per hour.

The maximum S.D.A. for the high-protein diet is 33.0 cal. per hour at 2 hours post prandium, decreasing to 2.7 cal. per hour at the 12th hour post prandium, and to 1.0 cal. at the 16th hour. For the high-carbohydrate diet, the rate of extra caloric production is 21.0 cal. per hour at the peak 1.5 hours post prandium, decreasing to 1.3 at 12 hours and to 0.46 cal. at 16 hours.

The rate of extra calorie production is very small on both diets at 12 hours post prandium and quite insignificant at 16 hours. Taking the latter period for the high-protein diet, and the former for the high-carbohydrate diet as the period of active food absorption, we may estimate the *total* S.D.A. for the high-protein diet as 169 cal. and for the high-carbohydrate diet as 103 cal. Since the average caloric intake was 993 cal. on the high-protein diet, and 1070 cal. on the high-carbohydrate diet (see table 1), the total S.D.A. expressed in the usual terms is 17.0% of the ingested calories for the high-pro-

tein diet, and 9.6% for the high-carbohydrate diet. These values are higher than those of Benedict and Carpenter ('18), i.e., 12% and 6%, respectively, because they cover the entire absorptive period.

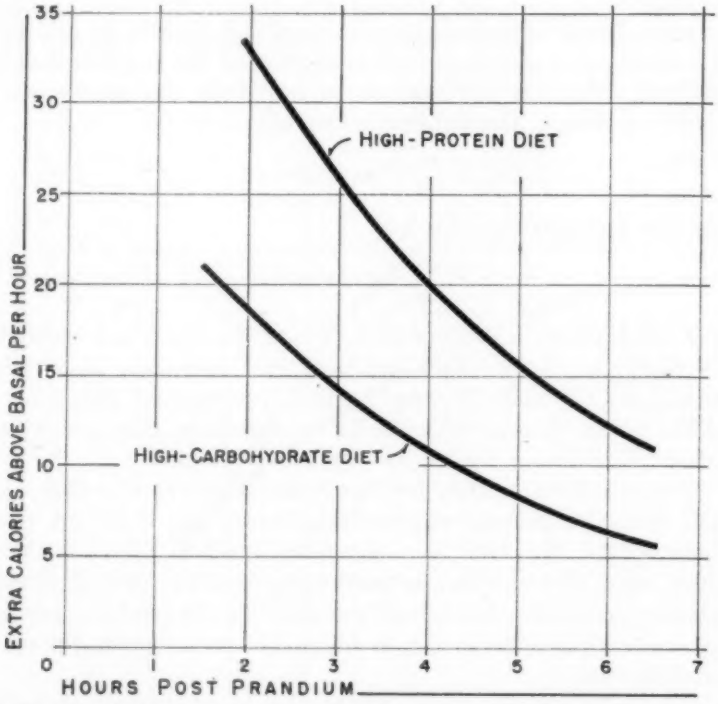

Fig. 2 The rate of extra calories production per hour after high-protein and high-carbohydrate meals.

The variation among individual subjects in the cumulative S.D.A. may be assessed from the total extra calories at the end of 6.5 hours post prandium. The coefficients of variation for the high-protein meals were 19% and for the carbohydrate meals, 16%, pooling in each case the results for the comfortable and the cool room. In the experiments of Wachholder and Franz ('44), the coefficients of variation in the total ob-

served S.D.A.'s for the different meals varied from 15 to 41%, averaging 28%.

DISCUSSION

The accumulation of extra calories above a base-line expenditure after a meal pursues a sigmoid course when plotted against time post prandium, i.e., the rate of accumulation increases for a period of 1.5 to 2.5 hours and then decreases until the metabolic stimulus, whatever it may be, is exhausted. The nature of the stimulus (or stimuli) is not known, but one may look at the situation from the standpoint of the plethora theory, championed and then abandoned by Lusk. Regardless of its validity, it does explain reasonably and more completely the observed facts concerning the specific dynamic action of nutrients and the factors affecting it than any other theory yet proposed.

According to the theory of plethora, the end products of digestion slowly establish a plethora in the tissues [3] as long as the rate of inflow exceeds the rate of tissue utilization for purposes of oxidation or of synthesis. During this phase of the phenomenon, the establishment of a plethora, and consequently the S.D.A., proceeds at a self-accelerating rate. As the rate of inflow diminishes (and the rate of utilization possibly increases) the plethora subsides and the rate of S.D.A. decreases. That this decrease should follow the course of the law of diminishing returns is not surprising, since the slower and slower inflow of each successive unit of nutrients into the tissues would be expected to induce a smaller and smaller calorigenic response. The theory can accommodate the observed facts concerning the participation of the endocrine glands in the S.D.A. of food in so far as hor-

[3] Arguments advanced against the theory too often assume that the plethora of nutrients in the tissues parallels that in the blood. When the rate of S.D.A. does not parallel the changes in nutrient concentration in the blood, the conclusion is drawn that the theory is invalid. However, there is no reason to believe that tissue concentration of nutrients and blood concentration run parallel in the post-absorptive period; for the amino acids, this has been known not to be true, since the classic studies of Van Slyke and Meyer ('13).

mones may modify the response of tissues to a calorigenic stimulus, i.e., their irritability (Mitchell, '27). Extrapolation of the curve expressing the relationship of cumulative S.D.A. and hours post prandium to the time of termination of the S.D.A. stimulus may be justified on the basis of the reasoning just discussed, as well as on the basis of the goodness of fit of the data to the mathematical expressions employed to describe them.

Above environmental temperatures setting the lower limits to the zone of thermic neutrality in the fasting man, the S.D.A. may reasonably be regarded as a wastage of food energy and even a burden upon the temperature-regulating mechanism. Below such temperatures it may serve a useful physiological purpose by sparing body tissues and increasing the tolerance to cold. But in either case it is a small item in the energy economy of man. After a high-protein meal containing approximately 1000 cal., the maximum rate of extra calorie production is 33 cal. per hour, decreasing in 5 and 6 hours, the usual period between daytime meals, to 15 and 12 cal. per hour, respectively (see fig. 2). After high-carbohydrate meals, the maximum rate is 21 cal. per hour, and the rates after 5 and 6 hours are 8 and 6 cal., respectively. Of the many human activities for which Sherman ('45, table 23) gives the caloric equivalent per hour, there is none, when allowance is made for basal heat expenditure, with as low a caloric equivalent as the S.D.A., even of a high-protein meal. Thus, the extra calories over basal for standing relaxed are 40 per hour; for hand sewing, 46 per hour; for dressing and undressing, 53 per hour; and for typewriting rapidly, 75 per hour.

The difference in S.D.A. between a high-protein and a low-protein meal of 1000 cal. is even less significant, starting at 12 cal. per hour at the peak, and diminishing to 8 and 6 cal. per hour, respectively, at 5 and 6 hours post prandium. It is not surprising, therefore, that Pitts, Consolazio and Johnson ('44) were unable to detect any appreciable increase in the thermal load of men working in a hot environment induced by an intake of 150 gm of protein daily, as compared with

an intake of 75 gm, the caloric intake remaining the same. Also, Keeton and coworkers ('46) found that the higher S.D.A. of a high-protein diet was not the determining factor in its effect upon tolerance to an intensely cold environment. Furthermore, Robinson and Lee ('47), working with domestic animals, did not secure any evidence that a high proportion of protein in the ration had any significant effect upon the reactions of the animals to heat.

SUMMARY AND CONCLUSIONS

The specific dynamic action of 2 diets containing 7 and 37% of protein calories was measured for 6 young men in each case, for periods of 6 to 7 hours post prandium, both in a comfortable and in a cool room. The rate of accumulation of extra heat above the fasting sitting metabolism after meals containing about 1000 cal. was found to follow a sigmoid course, the accelerating phase terminating at about 1.5 hours post prandium for the high-carbohydrate (low-protein) meals, and at about 2 to 2.5 hours post prandium for the high-protein meals. Thereafter, the rate of accumulation of extra calories followed the equation expressing the law of diminishing returns.

From equations of the latter type fitted to the experimental data, pooled for the 2 environments, the total S.D.A. was calculated, the results being 169 cal. for the high-protein meals and 103 cal. for the high-carbohydrate meals, or 17.0 and 9.6%, respectively, of the total calories consumed.

From these equations the hourly rate of extra calorie production was estimated on the basis of the corresponding differential equations. Comparing these rates with even the lightest types of muscular activity of men, it is evident that the S.D.A. of food is an inconsiderable item in the energy metabolism of active men, and in particular that high-protein meals as compared with low-protein meals exert an inappreciable effect in enhancing the thermal load of men working in a hot environment, or in protecting men against a cold environment.

ACKNOWLEDGMENTS

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THE NATURE OF THE SUPPLEMENTARY VALUE OF THE PROTEINS IN MILLED CORN MEAL AND MILLED WHEAT FLOUR WITH DRIED FOOD YEASTS[1]

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In recent publications (Sure, '46, '47) it was reported that when 1, 3 and 5% of milled enriched flour or milled white corn meal[2] are replaced by equivalent amounts of dried brewers' yeast (K) or a primary-grown cultured yeast (G)[3], there resulted large gains of body weight and marked increases in protein efficiency ratios, as evident from gains in weight per gram of protein intake. Since Mitchell and Smuts ('32) found that the proteins of wheat are deficient in lysine and the proteins of corn are deficient in lysine and tryptophane, it was anticipated that the dried food yeasts contributed these amino acids in the protein supplementation of these cereal grains. However, part of the marked responses to the administration of the yeasts may have been due to the influence of other dietary essentials. In this investigation an attempt was made to determine to what extent lysine could supplement for growth the proteins in milled enriched wheat

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[2] This was a "white table corn meal," purchased in local grocery stores.

[3] Furnished by Anheuser-Busch, St. Louis, Mo.

flour, and lysine and tryptophane the proteins in milled corn meal, as compared with different amounts of cultured yeast (G) added to the rations. All the experimental animals were kept on the same protein level. However, the rats which had supplementary administrations of lysine and/or tryptophane received additional small amounts of nitrogen furnished by these amino acids.

The terms "proteins in wheat" and "proteins in corn meal" are used here to mean the total proteins as they exist in the natural state in these cereal grains. The total protein was calculated from the nitrogen content found by analyses.

This study was conducted on Wistar strain albino rats, according to a technique recently described (Sure, '46, '47). The basal rations contained 89% wheat flour or 89% corn meal as sources of proteins, 2% cellu flour for roughage, and 4% of Sure's salt mixture no. 1 (Sure, '41). When a certain proportion of yeast was added to the rations, enough wheat flour or corn meal was removed to keep the protein content the same. Two per cent cod liver oil and 3% wheat germ oil in the rations furnished the fat-soluble vitamins. The following components of the vitamin B complex were administered to each animal daily separately from the rations: 100 μg thiamine, 100 μg riboflavin, 100 μg pyridoxine, 100 μg nicotinic acid, 600 μg calcium pantothenate, 12 mg choline chloride, 12 mg p-aminobenzoic acid, and 3 mg inositol. The results of this study are summarized in table 1.

The work with the wheat flour was carried out for a period of 10 weeks and it was intended to conduct the experiments on the corn meal rations for an equal period. However, the rats on the unsupplemented corn meal rations began to fail completely after 6 weeks; hence, it was necessary to terminate this study earlier. The animals on the corn meal rations were, therefore, kept on experiments for only 6 weeks.

In table 1 are shown the results of additions of 1, 3 and 5% cultured yeast (G) to the enriched wheat flour rations. The animals on rations containing 89% enriched wheat flour served as the controls. The latter ration was also supple-

TABLE 1

Influence on growth[1] and protein utilization of additions of lysine or tryptophane, or both, and cultured food yeast (G) to the proteins in enriched flour and in milled corn meal. All data are averages

TYPE OF RATION	PER CENT IN RATION	NUMBER OF ANIMALS	DAILY INTAKE				GAIN IN BODY WEIGHT	INCREASE IN BODY WEIGHT	TOTAL FOOD INTAKE	TOTAL PROTEIN INTAKE	PROTEIN EFFICIENCY RATIO[2]	INCREASE
			Lysine	Tryptophane	Lysine from yeast	Tryptophane from yeast						
			mg	*mg*	*mg*	*mg*	*gm*	%	*gm*	*gm*		%
Enriched flour	89.0	30	..	..	..	...	28.1	...	348	31.9	0.88	...
Enriched flour Cultured yeast (G)	85.2 1.0	24	..	..	1.0	...	39.2	39.5	385	35.3	1.11	26.1
Enriched flour Cultured yeast (G)	77.7 3.0	24	..	..	3.1	...	51.0	81.5	385	35.3	1.44	63.6
Enriched flour Cultured yeast (G)	70.2 5.0	24	..	..	5.1	...	62.1	121.0	387	35.5	1.75	98.8
Enriched flour	89.0	24	8	..	..	...	43.5	54.8	389	35.7	1.22	38.6
Enriched flour	89.0	30	20	..	..	...	53.2	89.3	400	36.7	1.45	64.8
Enriched flour	89.0	24	40	..	..	...	60.4	114.9	411	37.7	1.60	81.8
Enriched flour	89.0	24	60	..	..	...	69.5	147.3	407	37.3	1.86	111.3
Corn meal	89.0	24	..	..	..	...	30.4	...	314	25.0	1.21	...
Corn meal Cultured yeast (G)	76.3 3.0	24	..	..	3.1	0.54	48.6	+60.0	315	25.1	1.93	59.5
Corn meal Cultured yeast (G)	67.9 5.0	24	..	..	5.1	0.90	60.6	+99.3	362	28.9	2.09	72.7
Corn meal	89.0	12	20	..	..	...	25.1	—17.4	272	21.7	1.16	...
Corn meal	89.0	12		10	..	...	33.5	+10.2	349	27.8	1.21	0.0
Corn meal	89.0	24	20	10	..	...	68.1	+124.0	407	32.4	2.10	73.5

[1] Changes in body weight.

[2] Gain in weight per gram of protein intake.

Note: The experimental period for the animals on the enriched flour rations was 10 weeks; for those on the corn meal rations, 6 weeks. The total protein content of each ration for the first groups of rats (flour rations) was 9.17%; for the second groups (corn meal rations) 7.97%.

mented daily with 10, 25, 50 and 75 mg lysine hydrochloride, which in terms of pure lysine, corresponded to 8, 20, 40 and 60 mg, respectively. These amounts of amino acids furnished each rat daily approximately 1.6, 4, 8 and 12 mg supplementary nitrogen, respectively.

It will be noted that optimum growth and greatest protein efficiency were obtained on the 60 mg lysine supplementation and on the 5% yeast addition to the ration. However, the enriched flour ration, supplemented with 20 mg lysine daily, produced gains in body weight and protein efficiency similar to those on the ration containing 3% of the G yeast. The latter ration, however, furnished only 3.1 mg lysine daily.[4] It will also be noted that the enriched flour ration supplemented with 60 mg lysine produced a protein efficiency ratio of 1.86, which is very close to that produced by the 5% yeast-containing ration, which is 1.75; and the latter ration supplied only 5.1 mg lysine daily. The basal ration supplemented with 8 mg lysine daily produced a protein efficiency ratio of only 1.22. It would appear then that the additions of 3 and 5% of the G yeast, which supplied lower intakes than 8 mg lysine daily, stimulated growth by virtue of the addition of one or more other dietary essentials. One of these may be the amino acid, valine, since Light and Frey ('43) found that the proteins of wheat are deficient in valine as well as lysine. Another possible explanation is that the yeast may have furnished one or more of the as yet unidentified components of the vitamin B complex.

It is also apparent from table 1 that neither lysine nor tryptophane added alone improved the biological value of the proteins in milled corn meal. As a matter of fact, the addition of lysine alone reduced food consumption and lowered the protein efficiency ratio compared with results on the unsupplemented basal ration. However, the daily administration of 20 mg lysine and 10 mg tryptophane (which furnished about 5.5 mg of supplementary nitrogen) was accompanied by in-

[4] The lysine and tryptophane contents of the G yeast were determined microbiologically at the laboratories of Anheuser-Busch, St. Louis, Mo.

creases in growth and protein efficiency similar to those characteristic of the ration containing 5% of the G yeast. The increased growth on the ration supplemented with these amino acids is due to increased food intakes. Since, however, the 5% yeast-containing ration supplied daily only 5.1 mg lysine and 0.9 mg tryptophane, the yeast must have furnished an additional dietary factor or factors to account for such gains in body weight with such low intakes of these amino acids.

SUMMARY

The increased biological value of the proteins in milled enriched wheat flour with dried food yeast (G) is due to the latter's provision of the amino acid, lysine, and also possibly to one or more other dietary essentials contained therein.

The increased biological value of the proteins in milled white corn meal with dried food yeast (G) is due to the latter's provision of the amino acids, lysine and tryptophane, and also possibly to one or more other dietary essentials contained therein.

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RELATIVE SUPPLEMENTARY VALUES OF DRIED FOOD YEASTS, SOYBEAN FLOUR, PEANUT MEAL, DRIED NON-FAT MILK SOLIDS, AND DRIED BUTTERMILK TO THE PROTEINS IN MILLED WHITE CORN MEAL AND MILLED ENRICHED WHEAT FLOUR [1]

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In recent studies (Sure, '46, '47) it was shown that when as little as 1 and 3% of dried brewers' yeast (K) or a dried cultured food yeast (G) or soybean flour replaced equivalent amounts of milled white corn meal or milled enriched wheat flour, there resulted pronounced increased growth and increases in protein efficiency ratios, as evident from gains in weight per gram of protein intake. Since the protein content of the yeasts was 45 to 50% and that of the soybean flour about 50%, the addition of even small amounts of these high-protein-containing foods produced appreciable increases in the total protein content of the rations. Therefore, the previous investigations dealt essentially with protein enrichment. In this investigation results are submitted on the relative

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supplementary values of several high-protein-containing foods to the total proteins as found in the natural state in milled white corn meal and in milled enriched wheat flour. No proteins were isolated from any of the foods used but their total protein content was calculated from nitrogen analyses.

Different batches of wheat flour, corn meal, non-fat milk solids and dried sweet-cream buttermilk varied in the total nitrogen content. However, in the construction of the rations, the necessary adjustments were made in the proportions of the high-protein-containing food supplements. In order to calculate the total protein, the conventional factor of 6.25 was not always used, but the different factors for various foods, as recommended by D. B. Jones ('31) were employed, i.e., 5.7 for wheat, 6.25 for corn, yeast, soybean flour, and 6.38 for the dried milk solids. Yeasts contain non-protein nitrogen, such as purines and pyrimidines. For this reason a minimum deduction of 10% was made in calculating the protein content of the yeasts.[2] Thus, the true protein content of the G and K yeasts used in this study is represented by 90% of nitrogen $\times$ 6.25.

All the rations[3] were so constructed that they contained the same total protein in the basal corn meal or wheat rations as those containing the various supplements. The amounts of soybean flour, peanut meal, dried non-fat milk solids, and dried sweet-cream buttermilk were added in such proportions that they furnished the same amounts of protein as those supplied by the G and K yeasts; and the total protein contents of all these rations were the same. Therefore, the results secured can be interpreted as true supplementary values.

[2] Personal communication from H. E. Carter, Department of Biochemistry, University of Illinois.

[3] The dried food yeasts were furnished by Anheuser-Busch, St. Louis, Mo. The soybean flour, processed and solvent-extracted, was supplied by Archer-Daniels Company, Minneapolis, Minn. The peanut meal was a processed product, sold under the trade name of "peanut granules," and supplied by Trades Oil Mill Co., Fort Worth, Texas. The dried non-fat milk solids and dried sweet-cream buttermilk were spray-processed Kraft and Borden products.

This study was carried out on Wistar strain albino rats. The composition of the rations is given in a previous paper (Sure, '48). Each animal on the 89% corn meal and 89% enriched wheat flour rations had the following vitamin B complex daily supplement: 100 μg thiamine, 100 μg riboflavin, 100 μg pyridoxine, 100 μg nicotinic acid, 600 μg calcium pantothenate, 12 mg choline chloride, 12 mg p-aminobenzoic acid, and 3 mg inositol. Each rat which had the 60% corn meal and the 60% wheat flour rations received the following daily vitamin B complex supplement: 50 μg of each of the following: thiamine, riboflavin, pyridoxine, and nicotinic acid; 300 μg of calcium pantothenate, 9 mg choline chloride, 6 mg p-aminobenzoic acid, and 3 mg inositol. The results of this investigation are submitted in tables 1 and 2.

From table 1 it is evident that on the ration containing 89% corn meal, the total protein content of which was 7.97%, peanut meal proved the poorest supplement. This may be due to low food consumption which may have been influenced by lack of palatability and/or amino acid deficiencies of the peanut meal, since amino acid deficiencies, like vitamin deficiencies, produce inanition (Rose and Epstein, '39). There seem to be no noteworthy differences between cultured yeast (G), soybean flour, dried non-fat milk solids and dried sweet-cream buttermilk when fed as protein supplements to a ration containing 75% milled white corn meal.

On the ration containing 60% corn meal, the protein content of which was only 6.08%, there undoubtedly existed pronounced amino acid deficiencies; hence, peanut meal must have furnished supplementary amino acids to corn meal proteins, because it produced a definite supplementary effect. Nevertheless, peanut meal proved the poorest supplement on the lower level as well as on the higher level of corn meal intake, compared with the food yeast, soybean flour, and dried milk solids. Here again, no marked differences in the relative supplementary values were found between the food yeast, soybean flour, dried non-fat milk solids, and dried sweet-cream buttermilk.

TABLE 1

Relative supplementary values of cultured yeast (G), soybean flour, peanut meal, non-fat milk solids, and dried buttermilk, to the proteins in milled corn meal (average results per animal for a 6-week period)

TYPE OF RATION	CORN MEAL AND PROTEIN FOODS IN RATION	GAIN IN BODY WEIGHT	INCREASE IN BODY WEIGHT	TOTAL FOOD INTAKE	TOTAL PROTEIN INTAKE	PROTEIN EFFICIENCY RATIO [1]	INCREASE
	%	*gm*	%	*gm*	*gm*		%
Group I							
Corn meal	89.0	30.7	...	314	25.0	1.23	...
Corn meal Cultured yeast (G)	74.97 3.00	54.7	78.1	393	31.3	1.75	42.3
Corn meal Soybean flour	74.97 2.57	48.1	56.7	362	28.9	1.66	34.9
Corn meal Peanut meal	74.97 2.11	31.5	2.6	328	26.1	1.21	0.0
Corn meal Dried non-fat milk solids	74.97 3.56	47.8	55.7	366	29.1	1.64	33.2
Corn meal Dried buttermilk	74.97 3.70	50.0	62.8	369	29.3	1.70	39.0
Group II							
Corn meal	68.0	6.7	...	233	14.2	0.47	...
Corn meal Cultured yeast (G)	54.1 3.0	26.2	291	309	18.8	1.39	196
Corn meal Soybean flour	54.10 2.57	24.9	272	311	18.9	1.32	181
Corn meal Peanut meal	54.10 2.11	17.1	156	291	17.7	0.96	104
Corn meal Dried non-fat milk solids	54.10 3.56	23.8	255	269	16.4	1.45	208
Corn meal Dried buttermilk	54.10 3.70	22.9	242	262	15.9	1.44	206

[1] Gain in weight per gram of protein intake.

Note: There were 6 ♂ and 6 ♀ rats in each experiment in group I, and 12 ♂ and 12 ♀ rats in each experiment in group II. Every ration contained 7.97% protein in group I, and 6.08% protein in group II.

The results on relative supplementary values of dried food yeasts, soybean flour, peanut flour, and the dried milks to the proteins in milled enriched wheat flour are summarized in table 2. Enriched wheat flour was fed at 2 levels of intake with 2 and 3% dried yeast (G) as a supplement and the rest of the high-protein-containing foods were fed in proportions which supplied the same amounts of protein as the yeast. Enriched wheat flour was also fed at a 60% plane of intake with 2% dried brewers' yeast (K) added as a basis of comparison.

From table 2 it would appear that the dried buttermilk was the best supplement to enriched flour fed at a 89% level and that the non-fat milk solids was a close second. However, on a higher plane of food yeast intake and corresponding higher intake of the rest of the food supplements, the greatest increase in body weight was obtained on the dried buttermilk because of the greatest food intake. On very similar food intakes, however, the animals on the soybean flour gained considerably more in body weight and showed a higher protein efficiency ratio than the rats on the peanut meal. The animals on the dried yeast (G) equalled in protein efficiency ratio those on the dried buttermilk. But the outstanding result indicated in table 2 is the superior supplementary value obtained with the dried buttermilk in comparison with the non-fat milk solids. This is even more accentuated on the 60% enriched wheat flour intake (groups II and III, table 2). The inferiority of peanut meal as a supplement compared with the other high-protein-containing foods is also quite evident.

When this study was completed in 1947 the interpretation was first made that dried buttermilk probably contains an essential dietary factor that is deficient in non-fat milk solids. However, on examining the container of the dried non-fat milk solids [4] it was found that it was purchased in 1946, while the dried sweet-cream buttermilk [5] was obtained in 1947. Con-

[4] A Borden product.

[5] A Kraft product supplied by the American Dry Milk Institute.

Every ration contained 7.97% protein in group I, and 6.08% protein in group II.

TABLE 2

Relative supplementary values of cultured yeast (G), soybean flour, peanut meal, dried non-fat milk solids, and dried buttermilk, to the proteins in enriched flour

TYPE OF RATION	FLOUR AND PROTEIN FOODS IN RATION	NUMBER OF ANIMALS	GAIN IN BODY WEIGHT	INCREASE IN BODY WEIGHT	TOTAL FOOD INTAKE	TOTAL PROTEIN INTAKE	PROTEIN EFFICIENCY RATIO [1]	INCREASE
	%		*gm*	%	*gm*	*gm*		%
Group I								
Enriched flour	89.0	12 [2]	41.0	...	582	53.3	0.77	...
Enriched flour Cultured yeast (G)	81.3 2.0	12 [2]	80.0	95.1	715	65.5	1.22	58.5
Enriched flour Soybean flour	81.30 1.72	12 [2]	57.9	44.8	614	56.2	1.03	33.7
Enriched flour Peanut meal	81.30 1.41	12 [2]	55.2	38.0	613	56.1	0.98	27.3
Enriched flour Dried non-fat milk solids [3]	81.30 2.37	12 [2]	75.7	89.2	643	58.9	1.28	66.2
Enriched flour Dried buttermilk [4]	81.30 2.47	12 [2]	90.6	126.5	748	68.5	1.32	71.4
Group II								
Enriched flour	89.0	12 [2]	40.8	...	537	49.2	0.83	...
Enriched flour Cultured yeast (G)	77.54 3.00	12 [2]	79.6	95.1	644	59.1	1.35	62.6
Enriched flour Soybean flour	77.54 2.57	12 [2]	71.3	74.7	620	56.9	1.25	50.6
Enriched flour Peanut meal	77.54 2.11	12 [2]	55.4	11.3	616	56.5	0.98	18.1
Enriched flour Dried non-fat milk solids [3]	77.54 3.56	12 [2]	76.7	88.0	699	64.1	1.20	44.5
Enriched flour Dried buttermilk [4]	77.54 3.70	12 [2]	93.4	129.0	752	68.9	1.35	62.6
Group III								
Enriched flour	89.0	12 [2]	41.6	...	564	51.7	0.80	...

Group III								
Enriched flour	60.0	12 [2]	16.1	...	508	31.4	0.51	...
Enriched flour Brewers' yeast (K)	51.3 2.0	12 [2]	41.6	155.3	629	38.9	1.06	107.8
Enriched flour Soybean flour	51.3 1.8	12 [2]	31.4	95.0	569	35.2	0.89	74.5
Enriched flour Peanut meal	51.30 1.63	12 [2]	20.3	26.1	504	31.1	0.65	27.4
Enriched flour Dried non-fat milk solids [3]	51.30 2.64	12 [2]	41.0	154.6	609	37.6	1.09	113.7
Enriched flour Dried buttermilk [4]	51.30 2.60	12 [2]	54.3	237.3	665	41.1	1.32	158.8
Group IV								
Enriched flour	89.0	24	44.7	...	555	49.8	0.82	...
Enriched flour Dried non-fat milk solids [5]	76.68 3.50	24	83.3	86.3	654	58.8	1.42	73.2
Enriched flour Dried buttermilk [5]	76.68 3.90	24	72.0	61.1	574	51.6	1.40	70.7
Group V								
Enriched flour	60.0	12	23.2	...	505	30.6	0.76	...
Enriched flour Dried non-fat milk solids [5]	50.84 2.60	12	54.6	135.4	645	39.1	1.39	82.9
Enriched flour Dried buttermilk [5]	50.84 2.96	12	40.7	74.9	599	36.3	1.12	47.4

[1] Gain in weight per gram of protein intake.

[2] One-half males and one-half females.

[3] This was a Borden product supplied in 1946.

[4] This was a Kraft product supplied in 1947 by the American Dry Milk Institute.

[5] All the dried milks used by groups IV and V were 1947 Kraft products supplied by the American Dry Milk Institute.

Note: All the rations containing 89% enriched flour and those following in such groups (I, II, IV) contained 8.99% protein. The rations containing 60% enriched flour and those following in such groups (III, V) contained 6.06% protein.

sequently, it was decided to repeat the work on these dried milks with new 1947 products. This was particularly necessary, since Hodson and Krueger ('47) found that on storage non-fat milk solids lost arginine, histidine, methionine and tryptophane.

The findings with the 1947 products are summarized in table 2, under groups IV and V. This table shows that the animals on the 1947 dried non-fat milk solids consumed more food than the rats on the 1947 dried buttermilk and, therefore, gained more weight. On the lower level of enriched wheat flour intake there was a definite higher protein efficiency ratio in favor of the dried non-fat milk solids. On the other hand, on the higher level of enriched flour intake, the protein efficiency ratio was the same on the 2 kinds of dried milks. The different supplementary values secured with the 2 batches of these dried milk solids may be due to different temperatures of drying, since Fairbanks and Mitchell ('35) demonstrated that with higher temperatures of drying there may occur losses of cystine in dried milks. Certainly, there appears to be no superiority in the biological value of the proteins in 1947 processed dried sweet-cream buttermilk over that of 1947 processed dried non-fat milk solids, when used as supplements to the proteins in enriched wheat flour.

SUMMARY

When used as supplements to the proteins in milled white corn meal, there were found very little differences between dried food yeasts, soybean flour, non-fat milk solids, and dried sweet-cream buttermilk. Peanut meal proved to be the poorest supplement.

When used as supplements to the proteins in milled enriched wheat flour, cultured yeast (G) and brewers' yeast (K) proved to be superior supplements to soybean flour. Peanut meal was the poorest supplement. Results with dried non-fat milk solids and dried sweet-cream buttermilk were variable, perhaps due to having used products that may have been subjected to different temperatures of drying.

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MINERAL METABOLISM STUDIES IN DAIRY CATTLE

IV. EFFECTS OF MINERAL SUPPLEMENTATION OF THE PREPARTAL DIET UPON THE COMPOSITION OF THE BLOOD OF COWS AND THEIR CALVES AT PARTURITION [1]

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Much attention has recently been given to the importance both of the calf's nutrition during fetal life and of the composition of colostrum. In general, these studies have dealt with vitamin A (Thomas et al., '46; Spielman et al., '46a, '46b; Wise et al., '46a, '46b), vitamin D (Hibbs, '46; Eaton et al., '47a, '47b), and vitamin E (Parrish et al., '47a, '47b) in the prepartal diet and/or in the colostrum of the dam and the effects of various treatments upon the blood and liver picture of the dam and/or of the newborn calf. Other studies in this connection have been concerned with choline (Waugh et al., '47), riboflavin (Sutton and Kaeser, '47) and globulin (Hansen et al., '46; Hansen and Phillips, '47a, '47b). Luecke et al. ('47) have reported data on the levels of iron, copper, cobalt, carotene, vitamin A, riboflavin, pantothenic acid, nicotinic acid and thiamine in the colostrum of the bovine.

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Very few data are reported on the effects of minerals in the prepartal diet upon the blood picture of newborn calves. Several reports, however, show that the level of plasma calcium is higher in the calf at birth than in the dam (Meigs et al., '19; Robinson and Huffmann, '26; Theiler et al., '27; Godden and Allcroft, '32). Groenewald ('35), using a low-mineral prepartal diet, found a similar situation for calcium and other elements in the blood of the dam and her calf. Robinson and Huffman ('26) and Godden and Allcroft ('32) found greater concentrations of inorganic phosphorus in the calf than in the dam.

In view of the lack of data giving a broad blood picture, especially that of the same blood specimen, of the cow and her progeny at the time of parturition, it was the purpose of this investigation to study the effects of mineral supplementation upon a number of blood characteristics and constituents of the cow and her newborn calf, to determine whether sex influenced the blood picture of the newborn calf, and to study the effects of colostrum upon the composition of the blood of the newborn calf.

EXPERIMENTAL

Four groups of cows were fed the following mineral supplements in the concentrate feed during at least the last 2 months of gestation: group I, basal concentrate feed (commercial dry cow and fitting ration); group II, basal concentrate feed plus 2.5% of $CaCO_3$ (c.p.); group III, basal concentrate feed plus 3.0% of calcite flour [4] and group IV, basal concentrate feed plus 3.0% of Mico.[5] The average contents of calcium, phosphorus and manganese of these concentrate feeds on dry basis are shown in table 1. All cows received

[4] Calcite flour has the following average percentage composition: calcium, 33; magnesium, 2.6; manganese, 0.02; iron, 0.20; copper, 0.002 and zinc, 0.02. Product of the Limestone Products Corporation of America, Newton, New Jersey.

[5] Mico has the following average percentage composition: calcium, 33; magnesium, 2; manganese, 0.20; iron, 0.20; iodine, 0.045; copper, 0.025; zinc, 0.01 and cobalt, 0.002. Product of the Limestone Products Corporation of America, Newton, New Jersey.

the same hay and silage and pasture in season. The groups were composed of the following numbers of cows: group I, 12; group II, 9; group III, 14 and group IV, 14.

An attempt was made to obtain blood samples from both the cows and their calves immediately following parturition and prior to the calves' sucking. Most of the data were obtained on samples procured within about 1 hour subsequent to calving and before the calf had access to colostrum; in 7 cases, however, blood was not obtained until the calves were several hours old (estimated average age of 4.5 hours) and after they had had some colostrum. No colostrum-fed calves were over 8 hours old when bled.

TABLE 1

Average calcium, phosphorus and manganese contents of concentrate feeds

GROUP	Ca	P	Mn
	%	%	%
I	1.00	0.64	0.0063
II	1.87	0.64	0.0058
III	2.04	0.67	0.0068
IV	1.97	0.65	0.0112

The determinations made, and the particular chemical methods used to determine the levels of certain blood and plasma constituents are as follows: hemoglobin (Sanford, Sheard and Osterberg, '33); reduced, oxidized and total glutathione (Woodward and Fry, '32); calcium (Clark and Collip, '25); inorganic phosphorus (Fiske and Subbarow, '25); acid and alkaline phosphatase at pH 5.0 and 9.3, respectively (King and Armstrong,[6] '34); total plasma proteins, globulin and albumin (Looney and Walsh, '39); and ascorbic acid (Mindlin and Butler, '38) with certain modifications. The red blood cell count and packed red cell volume (hematocrit) were determined according to standard procedures and the mean

[6] Procedure modified by Wiese et al. ('39). Unit of phosphatase defined as 1 mg phenol liberated from $Na_2C_6H_5PO_4$ substrate in 30 minutes at 37°C. by 100 ml plasma.

corpuscular hemoglobin and volume were derived by calculation.

RESULTS AND DISCUSSION

The mean levels of various blood and plasma constituents of the cows and their progeny are shown by groups in table 2 and are summarized independently of groups in table 5. Although similar levels of hemoglobin and oxidized glutathione were found in the blood of cows and their calves, the blood of calves contained a greater number and was composed of a greater proportion of erythrocytes than that of the dams. The mean corpuscular hemoglobin and volume were correspondingly lower in the calves. In these animals, the whole blood contained markedly higher concentrations of reduced and total glutathione, and the plasma contained higher levels of ascorbic acid, acid and alkaline phosphatase, calcium and inorganic phosphorus than in the dams. The plasma of the cows, however, contained significantly greater quantities of total proteins, globulin and albumin than that of the calves. Of the differences between the mean levels of constituents in the blood or plasma of the dams and their calves, only the acid phosphatase for the groups receiving calcium carbonate (group II) and calcite flour [7] (group III), the inorganic phosphorus for group II, and the packed red cell volume for group III lacked mathematical significance at the 5% level; however, these differences (table 5) were significant between all dams and calves, disregarding group.

The concentrations of various blood and plasma constituents of the cows and of their calves were so similar that it seemed that calcium (groups II and III) and calcium with trace elements (group IV) were without effect upon the constituents of blood studied. It would appear that concentrate feeds containing about 1% calcium are as effective in the maintenance of the plasma calcium of both the dam and the calf as those containing 2% calcium when fed with good quality roughage. In this regard, Bodansky and Duff ('41)

[7] See footnote 4, page 76.

TABLE 2

Blood and plasma composition of dams and their newborn calves (mean ± standard error of the mean)

	CATEGORY OF INTEREST	GROUP I		GROUP II		GROUP III		GROUP IV	
		12 Dams	12 Calves	9 Dams	10 Calves	14 Dams	14 Calves	14 Dams	14 Calves
BLOOD	Hemoglobin (gm %)	12.88 ± 0.32	13.27 ± 0.60	13.53 ± 0.22	13.97 ± 0.45	13.22 ± 0.17	12.97 ± 0.47	13.55 ± 0.29	14.31 ± 0.40
	Red cells (millions/mm³)	6.72 ± 0.30	10.46 ± 0.70	6.82 ± 0.29	10.68 ± 0.74	7.61 ± 0.59	9.34 ± 0.39	7.29 ± 0.20	10.44 ± 0.57
	Hematocrit (%)	35.42 ± 1.15	41.77 ± 2.29	35.78 ± 0.95	42.54 ± 1.26	36.48 ± 0.67	39.19 ± 1.87	37.86 ± 1.15	45.92 ± 1.62
	Mean corpuscular Hb ($\mu\mu$g)	19.17	12.69	19.84	13.08	17.37	13.89	18.59	13.71
	Mean corpuscular volume (μ^3)	52.71	39.93	52.46	39.83	47.94	41.96	51.93	43.98
	Reduced glutathione (mg %)	41.51 ± 1.77	59.86 ± 3.21	37.65 ± 2.83	57.96 ± 3.38	32.87 ± 2.99	49.04 ± 5.87	40.81 ± 1.83	59.26 ± 2.99
	Oxidized glutathione (mg %)	5.72	5.61	7.36	9.30	9.47	7.06	5.17	7.46
	Total glutathione (mg %)	47.23 ± 2.03	65.47 ± 3.87	45.01 ± 4.16	67.26 ± 3.59	42.34 ± 2.81	56.10 ± 4.55	45.98 ± 1.93	66.72 ± 2.28
PLASMA	Calcium (mg %)	9.58 ± 0.24	11.84 ± 0.47	9.78 ± 0.69	11.43 ± 0.46	9.75 ± 0.31	12.33 ± 0.45	10.52 ± 0.94	12.65 ± 0.57
	Inorganic phosphorus (mg %)	5.31 ± 0.57	6.88 ± 0.29	5.60 ± 0.78	6.55 ± 0.29	5.36 ± 0.49	6.56 ± 0.24	4.26 ± 0.28	6.17 ± 0.23
	Phosphatase, acid (units/100 ml)	1.12 ± 0.19	2.36 ± 0.30	1.21 ± 0.15	2.11 ± 0.72	0.96 ± 0.15	2.36 ± 0.31	1.72 ± 0.37	1.88 ± 0.30
	Phosphatase, alkaline (units/100 ml)	3.36 ± 0.61	17.24 ± 3.75	5.10 ± 1.42	18.04 ± 4.19	4.97 ± 1.07	25.72 ± 5.11	4.73 ± 1.20	15.12 ± 2.83
	Total proteins (gm %)	7.25 ± 0.27	3.23 ± 0.16	6.78 ± 0.37	3.20 ± 0.43	7.01 ± 0.18	3.91 ± 0.40	7.06 ± 0.29	3.21 ± 0.18
	Albumin (gm %)	3.73	1.69	3.85	1.68	4.16	1.84	4.12	1.87
	Globulins (gm %)	3.52 ± 0.45	1.54 ± 0.19	2.93 ± 0.37	1.52 ± 0.36	2.85 ± 0.18	2.07 ± 0.32	2.94 ± 0.25	1.34 ± 0.06
	Ascorbic acid (mg %)	0.33 ± 0.08	0.64 ± 0.08	0.22 ± 0.04	0.48 ± 0.05	0.28 ± 0.05	0.59 ± 0.06	0.28 ± 0.05	0.50 ± 0.07

demonstrated that normal fetal growth and storage of calcium and phosphorus occur in rats despite great differences in the maternal intake of these elements, and concluded that the ability of the fetus to store calcium and phosphorus depends upon the maintenance of suitable concentrations of these elements in the blood on the maternal side and, probably also, on the fetal side of the placental barrier. Our data, in which a consistently higher level of calcium was found in the newborn calf than in the dam (average difference in plasma concentration, 2.19 mg %) as shown in tables 2 and 5, are not in agreement with those reported by Green and Macaskill ('28), who concluded that there are no characteristic differences between the blood of the calf and that of the dam relative to calcium content. The differences found in our study, however, are similar to those reported by Bogert and Plass ('23) for this element in the blood of the human mother and fetus.

The outstanding group differences observed were the lower levels of reduced and total glutathione of group III cows and calves than those of the respective animals of any other group. Although the calcite flour in the prepartal diet of these cows seemed responsible, the physiological significance of this difference is not known. These differences, though, were mathematically significant only between group III (calcite flour [8] supplemented) and groups I (basal diet) and IV (Mico [9] supplemented) for reduced glutathione in the dams' blood, and between group III and group IV for total glutathione in calves' blood.

With respect to the other constituents studied, it would appear that the basal ration (group I), and the basal ration supplemented individually by $CaCO_3$ (c.p.) (group II), calcite flour [8] (group III) and Mico [9] (group IV) were equivalent. Since the blood composition was not appreciably affected by the supplements used in this study, the data for the animals of all groups were combined and are presented in table 5.

[8] See footnote 4, page 76.
[9] See footnote 5, page 76.

The summarized data (table 5) for all cows compare favorably with those reported by Braun ('46) for cows (1.5 to 13 years old) on pasture.

Since the blood picture of mature bulls and cows is known to vary in a characteristic manner, the data for the calves were grouped in table 3 both according to sex and to the supplement fed their dams. These figures would indicate that the levels of the various constituents of blood studied were not influenced by the sex of the newborn calf or by the mineral supplement in the prepartal diet.

Many recent researches have been directed at the nutritive value of colostrum and have shown various effects of this food upon the composition (particularly vitamin substances) of the blood of the calf. In view of this and of the blood changes known to occur in calves during the first week of life (Wise et al., '47), a comparison was made of the blood picture of 7 calves which had received colostrum before blood was obtained, and 43 calves from which blood was taken immediately after birth and before these calves had suckled. These data are summarized in table 4 by groups including both sexes. Since mineral supplementation did not perceptibly influence the blood composition, the average blood picture for all calves, regardless of group, is shown in table 5. The data demonstrating the effects of colostrum did not appear to be related to sex; therefore, separate data for bull and heifer calves are not shown. The plasma levels of total proteins and globulin in the calves having had access to colostrum were strikingly higher than those of calves which were bled prior to nursing. The average age of the calves receiving colostrum was approximately 4.5 hours when the blood was procured for analyses.

Howe ('21) reported that the serum of the newborn calf before it has nursed does not contain proteins precipitable by 17.4% anhydrous Na_2SO_4 (i.e., blood of the newborn calf does not contain euglobulin or pseudoglobulin I). Within a few hours after the calf had received colostrum, Howe ('21) found large amounts of protein (euglobulin precipitable by 13.5%

TABLE 3

Blood and plasma composition of newborn bull and heifer calves (mean ± standard error of the mean)

	CATEGORY OF INTEREST	GROUP I		GROUP II		GROUP III		GROUP IV	
		6 Bulls	6 Heifers	5 Bulls	5 Heifers	6 Bulls	8 Heifers	9 Bulls	5 Heifers
BLOOD	Hemoglobin (gm %)	13.75 ± 0.74	12.69 ± 1.00	14.02 ± 0.80	13.91 ± 0.54	13.40 ± 0.30	12.65 ± 0.79	13.96 ± 0.61	14.88 ± 0.30
	Red cells (millions/mm³)	10.98 ± 1.07	9.94 ± 0.96	10.80 ± 0.81	10.55 ± 1.33	8.94 ± 0.42	9.54 ± 0.54	10.41 ± 0.69	10.48 ± 1.05
	Hematocrit (%)	43.02 ± 3.06	40.27 ± 3.71	41.98 ± 2.15	43.10 ± 1.55	38.91 ± 2.46	39.39 ± 2.85	45.09 ± 2.53	47.24 ± 1.34
	Mean corpuscular Hb (μμg)	12.52	12.77	12.98	13.18	14.99	13.26	13.41	14.20
	Mean corpuscular volume (μ³)	39.18	40.51	38.87	40.85	43.52	41.29	43.31	45.08
	Reduced glutathione (mg %)	62.17 ± 5.90	57.94 ± 3.62	52.32 ± 4.92	63.60 ± 3.36	41.50 ± 13.10	53.75 ± 5.07	61.40 ± 3.95	55.41 ± 2.76
	Oxidized glutathione (mg %)	4.54	6.50	8.22	9.04	8.20	7.15	5.82	10.41
	Total glutathione (mg %)	66.71 ± 6.57	64.44 ± 5.05	60.54 ± 5.37	72.64 ± 3.77	49.70 ± 8.25	60.90 ± 4.80	67.22 ± 3.43	65.82 ± 2.13
PLASMA	Calcium (mg %)	12.19 ± 0.86	11.48 ± 0.41	11.55 ± 0.53	11.32 ± 0.82	12.84 ± 0.91	12.01 ± 0.47	13.30 ± 0.78	11.49 ± 0.53
	Inorganic phosphorus (mg %)	6.87 ± 0.46	6.89 ± 0.40	6.35 ± 0.52	6.75 ± 0.41	6.49 ± 0.32	6.62 ± 0.36	6.25 ± 0.29	6.03 ± 0.41
	Phosphatase, acid (units/100 ml)	2.00 ± 0.27	2.60 ± 0.46	2.35 ± 0.40	1.87 ± 0.21	2.78 ± 0.62	2.09 ± 0.33	2.07 ± 0.40	1.57 ± 0.46
	Phosphatase, alkaline (units/100 ml)	18.31 ± 5.64	16.53 ± 5.40	9.36 ± 2.00	24.99 ± 5.79	29.94 ± 7.82	22.71 ± 7.06	17.80 ± 3.19	10.84 ± 5.14
	Total proteins (gm %)	2.95 ± 0.09	3.51 ± 0.28	3.55 ± 0.82	2.85 ± 0.33	4.34 ± 0.60	3.59 ± 0.54	3.25 ± 0.27	3.13 ± 0.21
	Albumin (gm %)	1.69	1.69	1.69	1.68	2.06	1.67	1.90	1.81
	Globulins (gm %)	1.26 ± 0.06	1.82 ± 0.35	1.86 ± 0.70	1.17 ± 0.16	2.28 ± 0.38	1.92 ± 0.50	1.35 ± 0.09	1.32 ± 0.11
	Ascorbic acid (mg %)	0.72 ± 0.13	0.56 ± 0.13	0.54 ± 0.07	0.42 ± 0.07	0.59 ± 0.13	0.60 ± 0.06	0.50 ± 0.11	0.51 ± 0.05

TABLE 4

Blood and plasma composition of newborn calves of both sexes as affected by colostrum feeding (mean ± standard error of the mean)

	CATEGORY OF INTEREST	GROUP I RECEIVING		GROUP II RECEIVING		GROUP III RECEIVING		GROUP IV RECEIVING	
		Colostrum	No colostrum	Colostrum	No colostrum	Colostrum	No colostrum	Colostrum	No colostrum
		2 Calves	10 Calves	1 Calf	9 Calves	4 Calves	10 Calves	No calves	14 Calves
BLOOD	Hemoglobin (gm %)	12.10 ± 0.12	13.53 ± 0.71	12.74	14.11 ± 0.48	11.97 ± 1.21	13.77 ± 0.43		14.31 ± 0.40
	Red cells (millions/mm³)	9.33 ± 0.87	10.74 ± 0.84	8.59	10.91 ± 0.78	8.80 ± 0.52	9.61 ± 0.51		10.44 ± 0.57
	Hematocrit (%)	36.71 ± 1.31	42.90 ± 2.66	38.51	42.99 ± 1.32	33.79 ± 3.45	41.35 ± 1.92		45.92 ± 1.62
	Mean corpuscular Hb (μμg)	12.97	12.60	14.83	12.93	13.60	14.33		13.71
	Mean corpuscular volume (μ³)	39.95	39.94	44.83	39.40	38.40	43.03		43.98
	Reduced glutathione (mg %)	58.82 ± 10.26	60.09 ± 3.59	57.36	58.03 ± 3.78	39.86 ± 9.62	53.12 ± 7.20		59.26 ± 2.99
	Oxidized glutathione (mg %)	7.92	5.10	3.68	10.01	6.20	6.99		7.46
	Total glutathione (mg %)	66.74 ± 14.56	65.19 ± 4.11	61.04	68.04 ± 4.04	46.06 ± 7.39	60.11 ± 5.31		66.72 ± 2.28
PLASMA	Calcium (mg %)	11.83 ± 0.37	11.84 ± 0.56	10.73	11.51 ± 0.51	11.63 ± 1.03	12.64 ± 0.46		12.65 ± 0.57
	Inorganic phosphorus (mg %)	7.04 ± 0.19	6.85 ± 0.82	5.90	6.62 ± 0.32	7.03 ± 0.48	6.38 ± 0.27		6.17 ± 0.23
	Phosphatase, acid (units/100 ml)	3.36 ± 0.98	2.11 ± 0.26	3.15	1.99 ± 0.22	2.68 ± 0.56	2.21 ± 0.39		1.88 ± 0.30
	Phosphatase, alkaline (units/100 ml)	12.21 ± 7.70	18.50 ± 4.40	12.60	18.72 ± 4.68	35.77 ± 11.69	22.37 ± 5.56		15.12 ± 2.83
	Total proteins (gm %)	4.21 ± 0.12	3.03 ± 0.11	6.67	2.81 ± 0.22	5.68 ± 0.84	3.21 ± 0.20		3.21 ± 0.18
	Albumin (gm %)	1.47	1.73	2.03	1.64	2.16	1.72		1.87
	Globulins (gm %)	2.74 ± 0.68	1.30 ± 0.06	4.64	1.17 ± 0.33	3.52 ± 0.60	1.49 ± 0.17		1.34 ± 0.06
	Ascorbic acid (mg %)	0.57 ± 0.05	0.66 ± 0.10	.46	0.48 ± 0.20	0.62 ± 0.18	0.58 ± 0.06		0.50 ± 0.07

Na_2SO_4 and pseudoglobulin I, precipitable by 17.5% Na_2SO_4). Ragsdale and Brody ('23) reported that neither globulin nor immune bodies are found in the newborn calf or in the newborn calf after the ingestion of normal milk; but, following the ingestion of colostrum, both are found in the blood serum. These investigators believed that globulin and immune bodies from the blood of the dam pass unchanged into the blood of the newborn

TABLE 5

Blood and plasma composition of cows and newborn calves immediately following parturition

CATEGORY OF INTEREST	ALL COWS (49)[1]	ALL CALVES (50)[1]	CALVES (43)[1] NO COLOSTRUM [2]	CALVES (7)[1] COLOSTRUM [3]
		Whole blood		
Hb (gm %)	13.29	13.61	13.87	12.12
R.B.C.C. (millions/mm³)	7.16	10.19	10.42	8.92
R.B.C.V. (packed cells) (%)	36.48	42.30	43.50	35.29
Mean corpuscular Hb ($\mu\mu$g)	18.56	13.36	13.31	13.59
Mean corpuscular volume (μ^3)	50.95	41.51	41.75	39.56
Reduced glutathione (mg %)	38.29	56.36	57.82	47.78
Oxidized glutathione (mg %)	6.79	7.08	7.21	6.33
Total glutathione (mg %)	45.08	63.44	65.03	54.11
		Blood plasma		
Ca (mg %)	9.93	12.12	12.21	11.55
Inorganic phosphorus (mg %)	5.08	6.53	6.47	6.87
Phosphatase, acid (units/100 ml)	1.23	2.17	2.03	2.94
Phosphatase, alkaline (units/100 ml)	4.57	19.09	18.31	24.05
Total proteins (gm %)	7.04	3.41	3.08	5.40
Albumin (gm %)	3.99	1.78	1.75	1.94
Globulins (gm %)	3.05	1.63	1.33	3.46
Ascorbic acid (mg %)	0.28	0.56	0.55	0.58

[1] Numbers in parentheses indicate number of animals in each group.

[2] Calves receiving no colostrum before blood was procured for analysis.

[3] Calves bled after colostrum ingestion and at an estimated average age of 4.5 hours.

calf through the mammary gland of the dam and through the alimentary tract of the calf. Ledingham ('07) showed that during infectious diseases when the immune body content of the blood increases, there is a corresponding rise in the blood globulin level (the euglobulin fraction showing greater change than the pseudoglobulin fraction). According to Little and Orcutt ('22), calves not fed colostrum at birth are without agglutinins, but agglutinins appear in the blood within 1 hour and reach a maximum within 5 hours subsequent to the ingestion of colostrum. This would suggest a rapid rate of absorption. The study of Ragsdale and Brody ('23) indicated that colostrum contains 6–12% globulin. Crowther and Raistrick ('16) suggested that serum globulin is probably identical with colostral globulin, although Smith ('46), using more refined techniques, has pointed out that the 2 globulins are not exactly identical. Recently Hansen et al. ('46) found the gamma globulin component of calves' serum to be extremely low at birth, but within 18 hours after the ingestion of colostrum the gamma globulin fraction was increased to about 10% of the serum proteins.

A tendency towards a lower level of hemoglobin and a lower erythrocyte count and red blood cell volume was observed in calves having ingested colostrum than in those not having had this food (tables 4 and 5). These observations may have been the result of an increased blood dilution in the calves which had access to colostrum. Since blood was obtained from these calves when their average age was approximately 4.5 hours, it would appear that colostral globulin reaches the blood stream soon after ingestion. Perhaps an increased blood volume (or dilution) is a sequel to the increased plasma protein concentration. Contrary to the results of some of the early studies, the data shown in tables 4 and 5 would indicate the presence of small quantities of globulins [precipitable by half-saturated $(NH_4)_2SO_4$] in the newborn calf prior to the ingestion of colostrum.

SUMMARY

A study was made of the effects of calcium and of calcium with various trace elements in the prepartal diet upon the composition of the blood of 50 calves and their 49 dams immediately following parturition.

Mineral supplements fed for at least 2 months prepartum did not elicit perceptible effects upon the levels of calcium and inorganic phosphorus in the plasma of the newborn calves or of their dams. Of a number of whole blood and plasma constituents studied, the outstanding group differences found were the lower levels of reduced and total glutathione in both the cows and calves of group III (calcite flour [10] supplemented) than in those of the other groups (basal diet alone, calcium carbonate supplemented, Mico [11] supplemented).

Greater concentrations of reduced and total glutathione, calcium, inorganic phosphorus, acid and alkaline phosphatase, and ascorbic acid and a greater number of erythrocytes were found in the blood of newborn calves than in that of their dams; a greater corpuscular hemoglobin content, corpuscular volume, and a higher plasma level of total proteins, globulin and albumin were found in cows than in their calves immediately following parturition.

Similar concentrations of the various blood constituents studied were found, regardless of the sex of the newborn calf.

Seven calves which received colostrum prior to the procurement of blood for analyses at an average age of 4.5 hours had strikingly higher plasma levels of total protein, globulin and albumin than those which had not been nursed by their dams. The blood of the calves which had ingested colostrum contained slightly less hemoglobin, smaller numbers of erythrocytes and a smaller proportion of the whole blood volume consisting of erythrocytes than the other calves, which may have been due to dilution of the blood. Small quantities of globulin [precipitable by half-saturated $(NH_4)_2SO_4$] were

[10] See footnote 4, page 76.

[11] See footnote 5, page 76.

demonstrable in the plasma of newborn calves prior to the ingestion of colostrum.

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SELF SELECTION OF DIET

VIII. APPETITE FOR FATS [1]

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TWO FIGURES

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Deuel and Movitt ('44) reported that rats preferred diets containing butterfat to those containing corn oil, cottonseed oil, olive oil, peanut oil, soybean oil, or margarine. This preference was ascribed to the flavor of the butter. Parrish, Shimer and Hughes ('46), using the initial preference method of determining food choice, could find little evidence of a preference by rats for foods containing butterfat to those containing corn oil. It was shown in this laboratory that when rats were offered their choice of sucrose, hydrogenated vegetable oil, casein, and a salt mixture, a majority of animals selected fat to supply the largest part of their caloric requirement (Scott, '46).

The present report describes experiments in which other fats (corn oil, cottonseed oil, and butterfat) were used as choices in place of or in addition to the hydrogenated fat, as well as a selection experiment in which the fats were incorporated in mixed diets. This series completes a group of experiments in which proteins other than casein (Scott and Quint, '46) and carbohydrates other than sucrose (Scott and Verney, '47) were studied.

[1] Contribution no. 667 from the Department of Chemistry, University of Pittsburgh. Aided by grants of the Nutrition Foundation, Inc., and of the Buhl Foundation.

EXPERIMENTAL

In each of the first 3 experiments, 10 male and 10 female weanling rats were placed in individual cages and allowed to eat a mixed diet [2] offered in all of 4 cups. The experiments differed in that the sources of fats in the diets were different. Corn oil was used in the first experiment, alkali-refined cottonseed oil in the second, and butterfat in the third.[3] Vitamins were fed separately as pills.[4] During a 3-week control period, the amount eaten from each cup was recorded daily and the cups were then interchanged in a predetermined random manner. In the 3-week experimental period that followed, the choices given the animals were sucrose, casein, salt mixture, and the same fat as in the control period, each in a separate cup. The amount eaten of each was recorded and the cups interchanged daily as before.

In the fourth experiment, 20 male and 20 female weanling rats were divided into 4 groups such that each animal had 1 littermate of the same sex in each of the other 3 groups. The members of each group were fed one of the following mixed diets in all of 4 cups during the control period: (1) 10% hydrogenated vegetable oil [5] diet; (2) 10% corn oil diet; (3) 10% cottonseed oil diet; (4) 10% butterfat diet; with the other constituents the same as in the standard diet. In the experimental period all animals were given their choice of these 4 mixed diets in separate cups.

In the fifth experiment, each of 20 rats was placed in a cage with 6 cups, all of which contained a diet consisting of 24% casein, 62% sucrose, 4% salt mixture, and 2.5% each of hy-

[2] The mixed diet consisted of 62% sucrose, 10% fat, 4% salt mixture (Jones and Foster, '42) and 24% casein (Labco "vitamin-free").

[3] Corn oil was obtained from Corn Products Refining Company, cottonseed oil from Interstate Cotton Oil Refining Company, and butterfat by washing and drying a commercial sweet butter.

[4] One pill was given each rat daily. It contained approximately 60 μg thiamine hydrochloride, 120 μg riboflavin, 90 μg pyridoxine hydrochloride, 150 μg calcium pantothenate, 10 mg choline chloride, 1 mg α-tocopherol, and 55 I.U. vitamin A and 11 I.U. vitamin A as 0.001 ml Natola; all in a dextrin-powdered sugar base.

[5] "Primex."

drogenated vegetable oil, corn oil, cottonseed oil, and butterfat. During the experimental period, 7 choices were allowed the animals in separate cups. These were sucrose, casein, hydrogenated oil, corn oil, cottonseed oil, butterfat, and salt mixture.

RESULTS

The growth and food consumption of the animals in the 3-week control period are shown in tables 1 and 2. In the

TABLE 1

Growth and food consumption in the control period [1]

EXPERIMENT	FAT SOURCE	MALES		FEMALES	
		Wt. gain	Food eaten	Wt. gain	Food eaten
		gm	*gm*	*gm*	*gm*
1	Corn oil	83.6 ± 4.0	161.4 ± 7.7	74.5 ± 2.1	154.7 ± 4.7
2	Cottonseed oil	78.4 ± 4.5	156.4 ± 9.5	62.6 ± 3.0	136.3 ± 6.9
3	Butterfat	81.8 ± 4.8	167.4 ± 9.3	66.8 ± 3.1	146.2 ± 6.6
5	Mixture	47.5 ± 7.7	126.3 ± 11.7	43.3 ± 4.7	127.2 ± 8.9

[1] All data in terms of mean and standard error of the mean.

TABLE 2

Growth and food consumption during the control period (experiment 4)

FAT SOURCE	WT. GAIN	FOOD EATEN
	gm	*gm*
Hydrogenated fat	73.7 ± 4.1	155.9 ± 6.3
Corn oil	77.4 ± 4.0	155.0 ± 4.9
Cottonseed oil	62.0 ± 2.9	140.3 ± 6.9
Butter	71.0 ± 4.4	152.3 ± 8.1

7-choice experiment, the animals grew rather poorly for no obvious reason. Otherwise, the nutritional value of the fats, except that of cottonseed oil, appeared to be approximately equivalent. This was indicated by data on weight gains in the littermate experiment shown in table 2.

The choices of the animals during the control period in the first 3 and fifth experiments are shown in figure 1, while the

choice of fats in the fifth experiment is shown in figure 2. The mixed diets selected by the animals in the fourth experiment are summarized in table 3.

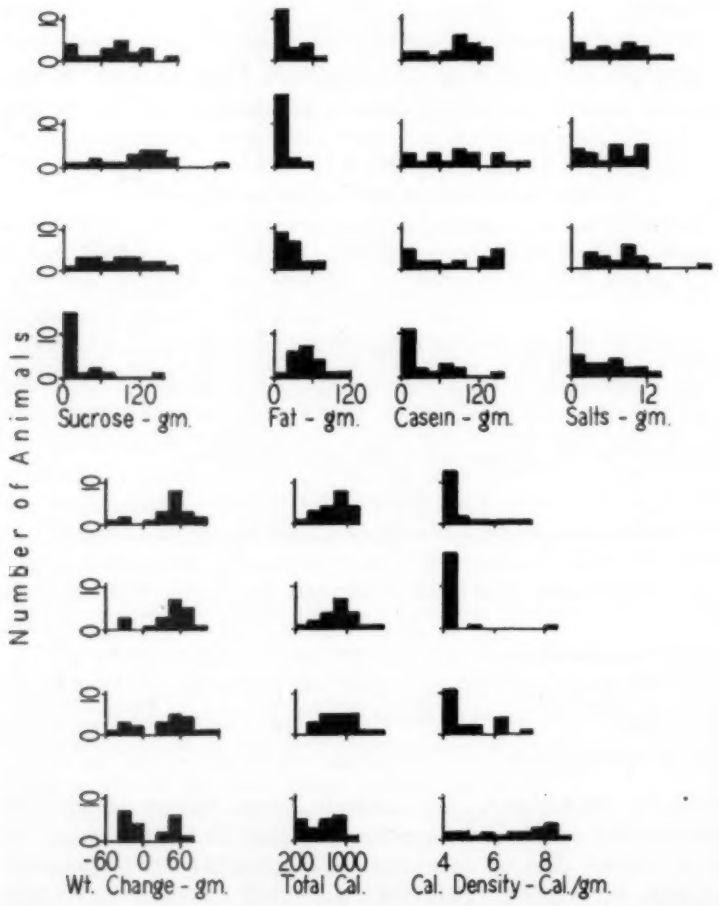

Fig. 1 Weight changes and food selections of rats during a 3-week period. The fat offered was in each case: Top histogram — corn oil; second histogram — cottonseed oil; third histogram — butterfat; bottom histogram — 4 fats simultaneously.

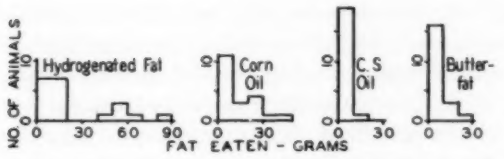

Fig. 2 Selection of fats when offered simultaneously.

DISCUSSION

In an earlier experiment (Scott, '46) where the choices offered were with respect to sucrose, salts, casein, and hydrogenated fat, 67% of the animals chose more fat in terms of calories than any other item, while 24% selected more sucrose, and 9% ate more protein. Hydrogenated fat thus being the food of choice, one might have expected more profound changes in the selection of other components if one offered a fat that was disliked than if one offered a protein or carbohydrate that was disliked.

In these experiments, none of the other fats was preferred as much as hydrogenated vegetable oil. The decreasing orders of preference were: 1 fat as choice, or fats incorporated in a diet — hydrogenated fat > butterfat > corn oil > cottonseed oil; 4 fats offered simultaneously — hydrogenated fat > corn oil > butterfat > cottonseed oil. These effects may have been due to the flavor of the fats and oils. Only the hydrogenated fat

TABLE 3

Appetite for mixed diets

DIETARY FAT SOURCE IN CONTROL PERIOD	WT. GAIN	FOOD EATEN	APPETITE FOR DIET CONTAINING [1]			
			Hydrogenated fat	Corn oil	Cottonseed oil	Butter
	gm.	*gm.*				
Hydrogenated fat	61.2 ± 7.7	226.1 ± 18.1	17.4 ± 9.2	— 7.4 ± 3.7	— 20.9 ± 3.0	11.2 ± 7.0
Corn oil	64.1 ± 7.6	235.5 ± 14.0	25.5 ± 8.7	— 5.3 ± 6.0	— 30.4 ± 7.1	9.7 ± 7.9
Cottonseed oil	75.0 ± 5.1	242.2 ± 7.7	24.5 ± 9.3	— 6.5 ± 7.3	— 13.6 ± 7.9	— 4.8 ± 10.4
Butter	77.0 ± 7.6	247.2 ± 14.2	31.5 ± 13.4	— 5.0 ± 9.0	— 15.8 ± 3.0	— 10.8 ± 7.7
Average	69.3 ± 3.5	237.7 ± 6.8	24.7 ± 5.0	— 6.0 ± 3.3	— 20.2 ± 2.9	1.4 ± 4.3

[1] Change in per cent eaten (experimental minus control) from corresponding cups during experimental and control periods. A positive change is indicative of a preference.

had an entirely bland flavor, and only this fat did not undergo flavor change on standing at room temperature. The cottonseed oil in particular became rancid quickly when incorporated in a diet. An attempt was made to keep fresh fats as choices. It was realized, however, that in any case the reaction of the animal to various degrees of rancidity or other flavor changes of the fats was not predictable.

The result of offering an unpopular fat such as corn or cottonseed oil as a choice was the selection by the animals of unusually large amounts of sucrose and casein. When several fats were offered as choices, little sucrose or casein was eaten.

These results and those obtained on other carbohydrates (Scott and Verney, '47) and on other proteins (Scott and Quint, '46) lead to 1 general conclusion: In this type of experiment, each food factor is selected or rejected on its own merits relative to the merits of the other possible choices. Accordingly, an item is selected by most animals if it is liked as well or better than the other possible choices and rejected by most animals if it is not liked as well. Such acceptance or rejection is generally not related to the nutritional nature of the choice, i.e., whether it is a carbohydrate, fat, or protein. One can increase the acceptability of a given choice by making other choices less acceptable or fewer in number, or decrease it by making them more acceptable or more numerous.

This interdependence of appetites is well illustrated in table 4, where data on acceptance from several experiments are compiled. Adopting a 20 to 1 level of significance, it will be noted that the only time less than the usual number of animals ate protein was when it was disliked (egg albumen); but more than the usual number ate it if several proteins were offered, or if the fat was unpopular (corn and cottonseed oils). Less than the usual number ate carbohydrate if it was disliked (lactose); but more ate it if it was well liked (starch), if more than 1 carbohydrate was offered, or if the fat offered was disliked (corn and cottonseed oils and butter). Less than the usual number selected fat if it was disliked (corn and cottonseed oils) or if several carbohydrates were offered.

There is little evidence in these studies that would indicate what properties of a given selected food item determine its acceptability for a rat. One could postulate that a pleasant flavor or texture, or the production of a subjective feeling of well-being would determine a favorable reaction, while an unpleasant flavor or texture, or unpleasant subjective symptoms, would have the opposite effect. In fact, there is no definite evidence in these studies that any factors other than

TABLE 4

Acceptance of various food choices by rats

CHOICES	NO. OF ANIMALS	ANIMALS SELECTING PROTEIN [1]	PROBABILITY [2]	ANIMALS SELECTING CARBOHYDRATE [3]	PROBABILITY [2]	ANIMALS SELECTING FAT [4]	PROBABILITY [2]
Casein, Sucrose, Primex [5]	87	53	1.000	43	1.000	76	1.000
Sucrose, Primex [6] and							
Lactalbumin	20	11	.777	7	.263	19	.490
Fibrin	20	13	.408	12	.503	17	.727
Egg albumen	20	6	.006	6	.126	20	.145
Four proteins	30	27	.010	18	.501	30	.140
Casein, Primex [7] and							
Starch	20	15	.214	16	.012	16	.290
Lactose	20	13	.777	0	< .001	20	.145
Dextrin	20	15	.214	14	.126	17	.727
Four carbohydrates	20	13	.777	19	< .001	13	.009
Casein, Sucrose and							
Corn oil	20	18	.010	16	.012	12	.002
Cottonseed oil	20	17	.036	19	< .001	8	< .001
Butterfat	20	14	.509	19	< .001	19	.490
Four fats	20	9	.152	6	.126	20	.145

[1] Number of animals that ate enough protein to gain weight over a 3-week period.

[2] Probability that, solely as a result of random sampling, a distribution of animals into groups that did or did not eat a particular choice could be as improbable as that found, provided the expected distribution into groups was that observed in the casein, sucrose, Primex experiment.

[3] Number of animals selecting at least 20 gm carbohydrate during a 3-week period.

[4] Number of animals selecting at least 10 gm fat during a 3-week period.

[5] Data taken from Scott ('46). Salt mixture was an additional choice in all cases.

[6] Data from Scott and Quint ('46).

[7] Data from Scott and Verney ('47).

the animals' subjective response to each group of choices are important in influencing selection. Because one cannot anticipate what the reaction of a given rat to a particular choice will be, the selections made under these conditions, while quite definite in some cases, often seem quite arbitrary. In consequence, the nature of selections from purified components of a diet did not appear to bear a demonstrable relation to the nutritional or physiologic requirements of the animals.

SUMMARY

Hydrogenated vegetable oil was more generally liked by young rats than butterfat or corn or cottonseed oils. When an unpopular fat was given as a choice, the rats selected much more casein and sucrose than when the choice was hydrogenated fat. It was concluded that choice of foods when components of a diet were offered was not related to the nutritional nature of the choices (i.e., whether a given choice was fat, carbohydrate, or protein), but was more probably dependent on the animals' subjective response to each particular choice.

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EFFECT OF PROTEIN LEVEL ON THE LYSINE REQUIREMENT OF THE CHICK

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FIVE FIGURES

(Received for publication February 2, 1948)

In the quantitative determination of the amino acid requirements of the chick, the level of protein has been kept at approximately 20% of the diet (Almquist, '47), but the effects of variation in the protein level are not known. An investigation of the problem should help in the exact definition of the amino acid requirements, and in the determination of the wisdom of feeding, at a higher dietary level, a protein slightly deficient in an essential amino acid in order to provide a satisfactory amount of the deficient amino acid in the diet.

It is well known that proteins slightly deficient in 1 amino acid can be fed in amounts which furnish more than the minimum level of protein, in order to satisfy the requirements for that particular amino acid. This is illustrated by the feeding of various levels of wheat gluten and other protein concentrates to rats (Barnes et al., '45), and by the feeding of 30% casein to satisfy the chick's arginine requirement, which is not met by 20% casein (Klose, Stokstad and Almquist, '38). Data are needed to determine the efficiency with which these high levels are used.

The lysine requirement was chosen because it has been well established by the use of several different proteins (Almquist, '47), and because there is a protein source available

(sesame meal) which, when fed at the 20% protein level, furnishes adequate amounts of all essential amino acids except lysine (Grau and Almquist, '44).

METHODS

White Leghorn chicks were reared on a stock diet for the first 2 weeks after hatching, then were segregated on the basis of weight into groups of 7 or 8 chicks, and given the experimental diets. The birds were housed in electrically heated battery brooders with wire floors; feed and water were supplied ad libitum. The chicks were weighed every other day during an 8-day period, and feed consumption was determined after 8 days. The basal diet consisted of calcium gluconate 8, tricalcium phosphate 2, sodium dihydrogen phosphate [1] 1, potassium chloride 0.6, sodium chloride 0.5, choline chloride [2] 0.2, inositol [3] 0.1, cholic acid [3] 0.1, crude soybean oil 5, mixed tocopherols [4] 0.05 and fortified sardine oil (3000A-400D per gm) 0.25 gm per 100 gm diet, and thiamine 1, riboflavin 1, pyridoxine 1, nicotinic acid 5, calcium (d) pantothenate 3, 2-methyl-1,4-naphthohydroquinone diacetate 1, biotin 0.1, pteroylglutamic acid [2] 1, manganese 10, silicon 46, magnesium 48, aluminum 8, iron 14, copper 1, zinc 1, iodine 0.8 and cobalt 0.5 mg per 100 gm diet. Sesame meal and L-lysine monohydrochloride monohydrate from commercial sources were added as desired, and glucose [5] was added to a total of 100 gm. The sesame meal contained 43.6% crude protein (N $\times$ 6.25).

[1] In some early experiments, dipotassium phosphate was used instead of sodium phosphate. In order to retain the same levels of potassium, phosphorus and sodium, changes were made in the levels of the chlorides.

[2] Provided by Lederle Laboratories Division, American Cyanamid Co., through the courtesy of Dr. T. H. Jukes.

[3] Present only in the early experimental diets; omitted after it was found to have no effect on growth.

[4] Concentrate of natural mixed tocopherols (15%), Distillation Products, Inc., Rochester, N. Y.

[5] Cerelose.

The growth rates were obtained by multiplying the average gain per chick per day by 100 and dividing by the average weight during the experiment, to give the per cent gain per day (Grau and Almquist, '43).

RESULTS

In figure 1, the growth rates for the various groups are plotted against the levels of supplementary lysine, at 4 protein levels. When only 5% protein was present, the best growth obtained was only about 1% per day, and this rate

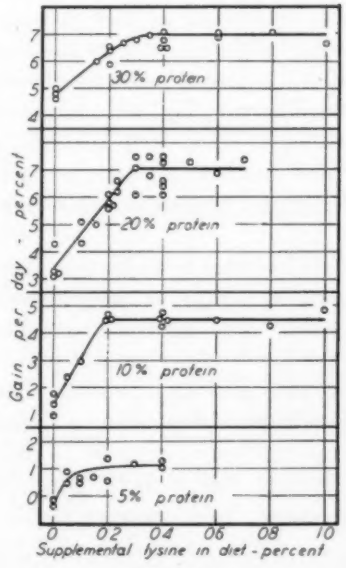

Figure 1

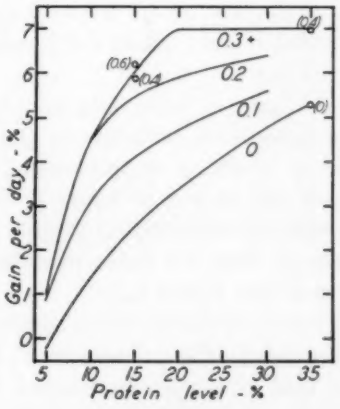

Figure 2

Fig. 1 Effects on growth of different levels of protein and of supplemental lysine. Each circle represents a group of 7 or 8 chicks, kept for 8 days on the diet after an initial period of 2 weeks on a control diet.

Fig. 2 Effects of protein level on growth rate, at various levels of supplemental lysine. The curves are drawn from the curves of figure 1. The circles represent additional experimental groups at protein levels other than those plotted in figure 1; their lysine levels are enclosed in parentheses. The other numbers are levels of supplemental lysine.

required the addition of approximately 0.1% lysine.[6] Although the point of inflection of this curve is not sharply defined, the rapid initial rise indicates that the minimum amount of lysine for this level of protein is approximately 0.1%. It is apparent that higher levels had no significant effect on the growth rate. The minimum supplemental lysine level for optimum growth with 10% protein is sharply defined at 0.2%; the growth rate was not affected by the feeding of higher levels of lysine. The 20% protein curve is established by groups from 6 different experiments, including 1 experiment reported previously (Grau, Kratzer and Asmundson, '46). While there was more variation at this protein level, the averages at any one lysine level fall close to the line drawn. The curve indicates that 0.3% added lysine was needed for maximum growth, which was 7% per day, as compared with 4.5% per day with 10% protein. With 30% protein, a growth rate of 4.7% per day was realized without supplemental lysine; about 0.3% added lysine supported maximum growth.

In figure 2, these data are plotted in order to show the effect on growth of variation in the protein supply at several constant levels of supplemental lysine. The curves are taken from the curves of figure 1, except for 4 experimental points which are added from groups fed 15% and 35% protein. Not enough data for these protein levels were available to define curves for figure 1.

Even without supplemental lysine, the growth rate increased as the protein level increased, in a manner similar to that observed by Barnes et al. ('45). Addition of 0.1% lysine increased the growth rate at all protein levels, but 0.2% lysine was effective only at protein levels above 5%. Similarly, 0.3% lysine was effective only when the protein level was above 10%; curves for lysine levels above 0.3% coincided with that of 0.3%.

[6] All data are calculated on the basis of lysine itself, although the monohydrochloride monohydrate was the form used.

Sesame meal protein contains 2.8% lysine (Block and Bolling, '45), so that the concentration of lysine in the basal diet is 0.14% at the 5% protein level; 0.28% at 10% protein; 0.56% at 20% protein; and 0.84% at 30% protein. When these basal levels are taken into account, the growth curves become oriented as shown in figure 3. These curves demonstrate clearly that when expressed in per cent of the diet needed to promote maximum growth, the lysine requirement increased

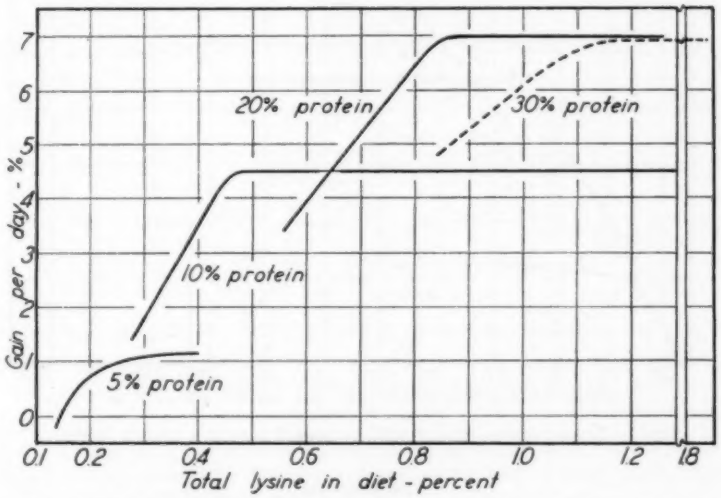

Fig. 3 Effects on growth of different levels of lysine and of protein. The curves are those of figure 1, with account taken of the lysine furnished by the sesame meal.

as the protein level increased, even at the 20% and 30% protein levels, where the maximum growth was also the best growth attainable by chicks under these conditions. The 20% protein level required 0.87% total lysine, which agrees remarkably well with the level (0.9%) originally set by Almquist and Mecchi ('42).

To ascertain whether or not the differences between protein levels were nullified by differences in feed consumption, actual

lysine intakes were calculated for each group. In figure 4, the results are plotted against the growth rate.[7] It is apparent that at any given growth rate below the maximum, more lysine is ingested at high, than at low protein levels.

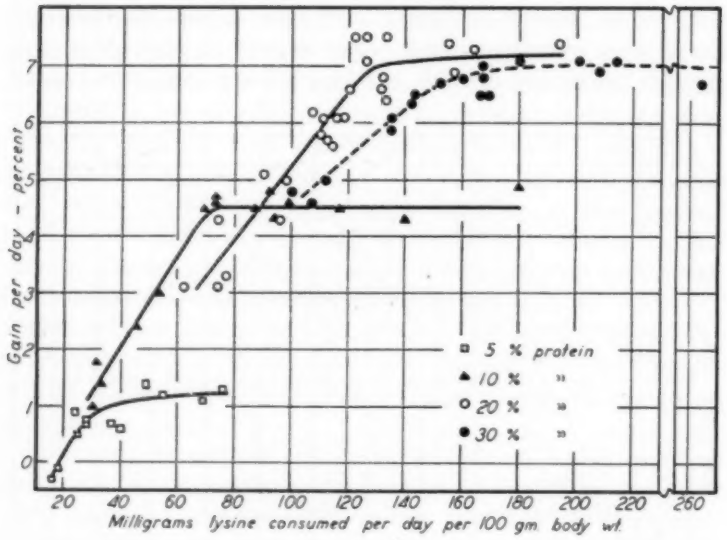

Fig. 4 Lysine consumption related to growth rate at 4 protein levels.

DISCUSSION

Since the conclusions drawn from this investigation are based primarily on growth rates of young animals, this basis for establishment of requirements should be justified. The strongest argument in favor of gain in weight as an adequate measure of protein and amino acid utilization is that there exists a constant tissue protein composition, regardless of the dietary level of an essential amino acid (Grau, '47). The question which remains is whether or not body weight is an

[7] The plot here is gain per 100 gm body weight (per cent gain) against lysine consumed per 100 gm body weight. Comparison of total gain against total lysine consumed presents the same general picture.

adequate measure of tissue protein content. Barnes et al. ('45) found that the maximum range of protein content of rat carcasses within a large range of protein levels and between proteins varying widely in value was "well within the experimental error of most biological assay methods with small numbers of animals." Results with chicks (Grau, '46) indicated that the phenylalanine level of the diet had no marked effect on the protein content of the carcass, and although poor diets produced carcasses with a low fat content, the effects

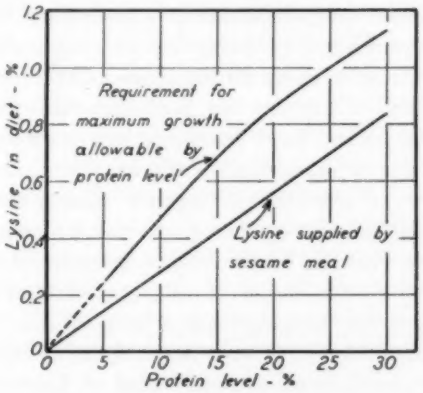

Fig. 5 The upper curve shows the relation of protein level to lysine requirement for maximum growth. The lower curve shows the level of lysine supplied by various levels of sesame meal protein.

on gain or loss of fat per day were negligible compared with gain or loss in weight.

In figure 5, the minimum lysine requirement for maximum growth which a particular level of protein will allow is plotted against the protein level, using the data from figure 3. Also shown in the same figure is the lysine supplied by various dietary levels of sesame meal protein. It should be noted that while the lysine requirement increases as the protein level is raised from 20% to 30%, this change causes no effect on the maximum growth rate (fig. 2).

The question of the availability of lysine in sesame meal must be considered as a possible cause for the results obtained. Except for the unlikely possibility that the proportion of available lysine varies with the protein intake, any effect of low digestibility of the protein or unavailability of the lysine would affect all protein levels by shifting the curves of figure 3 to the left. Some requirement difference for maximum growth would remain, however, because the curves have different slopes. Furthermore, the lysine requirement at the 20% protein level found here agrees very well with data obtained with other protein sources (Almquist, '47). Perhaps the strongest evidence in favor of the high availability of lysine in sesame meal rests on the slope of the curves at lysine levels below the minimum for best growth. Complete lack of an essential amino acid causes a loss in weight of about 3% per day under conditions such as these, and from this point the curve of growth rate against amino acid level rises along a straight line to optimum growth (Almquist, '47). If an appreciable portion of the lysine present in sesame meal were unavailable, the slope of these curves would have to be greater in order to approach a loss of 3% per day at 0 lysine. Extrapolation of the curves of figure 3 shows that they intersect the ordinate at growth rates of between —1.5 and —3% per day. Thus the level of lysine available to the chick must be close to the total calculated lysine level.

The results presented above indicate clearly that the lysine requirement must be defined in terms of the protein level of the diet. These results cannot yet be generalized to include all the other essential amino acids; however, the chick requirement for methionine has been found to increase as the protein level is increased.

A protein such as that of the sesame seed, which does not satisfy the lysine requirement when fed at the 20% protein level, may be fed at a higher level in order to approach the satisfaction of the requirements, but at the higher protein level the lysine requirement is higher; hence, the lysine will be less efficiently used, as will all the other amino acids.

The application of these results to the biological assay of lysine in proteins indicates that the addition of proteinaceous material containing an unknown amount of lysine to a diet deficient in lysine will give an estimate of the lysine content below the actual value. For example, if sesame meal is to be assayed, and the 20% protein curve of figure 3 serves as the basis of the assay, the growth obtained by adding 10% sesame meal protein will be the same as that obtained with the unsupplemented 30% protein level. When the observed growth rate (4.8%) is projected on the 20% curve and the lysine content of the diet is read off, the apparent lysine level of the diet will be found to be 0.67%. Since 20% sesame protein contains 0.56% lysine, 0.11% was apparently added by the 10% sesame protein. But 10% of sesame protein actually contains 0.28% lysine; thus the bioassay is in error by 0.17% of the diet, which is 61% of the actual amount. If the bioassay were correct, either the requirement for or the availability of the lysine, or both, would have to be much smaller. The arguments against these possibilities have been pointed out above.[8]

It has been reported recently that high casein levels increase the need for supplementary arginine in chick diets (Hill and Van Poucke, '47), but whether this effect is of the same type as that reported here cannot be stated until further experiments have been conducted.

SUMMARY

Various levels of sesame meal protein and lysine have been fed to young chicks, and the effects on growth and feed consumption have been observed. It was found that as the protein level was increased, the lysine requirement for maximum growth at a particular protein level increased. This was true whether the lysine requirement was expressed as percentage of the diet, or as the weight of lysine consumed per day per unit of body weight.

[8] Methionine bioassays showed no such discrepancy between chemical and biological methods of analysis when the protein level was kept constant (Grau and Almquist, '45).

Amino acid requirements may best be expressed in terms of percentages of the diet at a particular protein level, preferably the minimum protein level necessary to promote rapid growth.

While higher levels of sesame protein provide higher levels of lysine, this additional lysine is inefficiently used, because the requirement for it is also increased. Other amino acids are also inefficiently used, because the protein level is above the optimum.

A lysine bioassay procedure in which the amino acid content of proteinaceous material is determined by addition to a sesame meal diet will produce erroneous results unless corrections are made for the increased lysine requirement with increased protein levels.

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THE DETERMINATION OF THE MINIMUM NITROGEN REQUIREMENT OF THE ADULT DOG FOR MAINTENANCE OF NITROGEN BALANCE

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NINETEEN FIGURES

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Rose and Rice ('39) have shown that, in order to maintain positive nitrogen balance in adult dogs, a certain minimum quantity of each of the 9 essential amino acids must be administered daily. Thus, in part at least, a single indispensable amino acid, if present in smaller amount than the rest in relation to the quantitative requirements of the animal, may determine the utilization of a mixture. A well-known illustration of this fact is the increased growth of young rats obtainable by adding either cystine or methionine to diets in which less than an optimum amount of casein is the sole source of protein (Osborne and Mendel, '13; Cox, Mueller, Elman, Albanese, Kemmerer, Barton and Holt, '47). In this case, methionine is the amino acid limiting the utilization of this protein. Thus, the mixture supplying the amino acids in the respective proportions most nearly meeting the animal's quantitative requirements should maintain positive balance at the lowest level of nitrogen intake.

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With this in mind, experiments have been carried out to determine the minimum quantity of nitrogen which must be administered in the form of various proteins and hydrolysates to maintain positive nitrogen balance. The effect of adding methionine to casein and to an acid hydrolysate of casein has also been studied. Data so obtained make possible quantitative comparison of the efficiency of various amino acid sources for the maintenance of positive nitrogen balance. The protein, or mixture of amino acids, which maintains positive nitrogen balance at the lowest level of nitrogen administration probably contains the amino acids in proportions most nearly approximating the requirements of the animal. When the quantitative requirements of the species are known, the amount of nitrogen, in the form of various proteins or amino acid mixtures, necessary for maintenance will be an excellent measure of the biological value of the material.

The procedure and diet employed were the same as reported previously (Kade et al., '46). At all times approximately 75 cal. per kilogram of body weight were fed as a practically nitrogen-free diet, which contained approximately 60 mg of nitrogen per 100 gm of food, of which 40 mg originated in the vitamins added. Thus, the diet contributed approximately 10 mg of nitrogen per kilogram of body weight which was always taken into account in determining the total nitrogen fed. The amino acids were administered by incorporation in the food or separately by stomach tube. All test periods were 7 days, but the urine was analyzed daily. This was done primarily to follow the trend of nitrogen excretion. In order to eliminate the necessity of altering the quantity of nitrogen being fed as the weight of the animal varied, an optimum body weight at a nutritive state of 0.30 was estimated for each test dog by the Cowgill and Drabkin ('27) formula $W = (0.3L)^3$, where W = weight in kilograms and L = length in centimeters.

The following experiments gave the data presented in the accompanying graphs. Each graph represents a separate determination of the minimum quantity of nitrogen in the form

of a protein or hydrolysate necessary to maintain equilibrium. All the animals were in the same nutritive condition. Consistent and reproducible values can be obtained only when the dogs are carefully brought to this standard condition of minimum nitrogen metabolism. This is accomplished, as outlined in the previous paper, by feeding a 7% casein diet at a nitrogen intake of 150 mg per kilogram for several weeks.

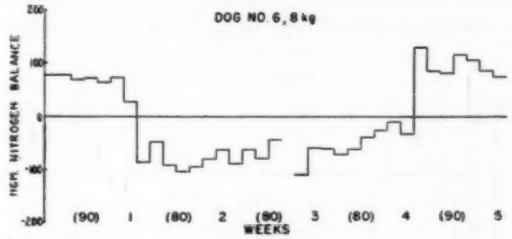

Figure 1

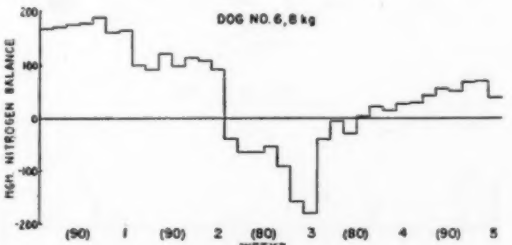

Figure 2

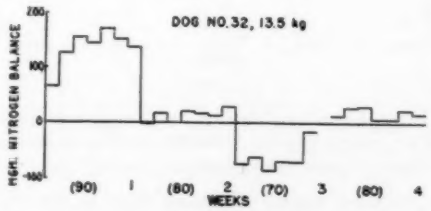

Figure 3

Figs. 1, 2 and 3 Nitrogen balance with lactalbumin as the sole source of amino acids. The figures in parentheses indicate the daily nitrogen intake per kilogram of body weight during that week.

DETERMINATION OF THE MINIMUM NITROGEN INTAKE NECESSARY FOR MAINTENANCE OF POSITIVE BALANCE IN THE ADULT DOG

Experiment 1. Lactalbumin.[2] The data presented in figures 1, 2 and 3 show that approximately 80 to 90 mg of nitrogen per kilogram of body weight, furnished as this lactalbumin, is sufficient for maintaining nitrogen balance in the adult dog.

Experiment 2. Blood fibrin.[3] The data of figures 4 and 5 show that approximately 100 to 110 mg of nitrogen per kilo-

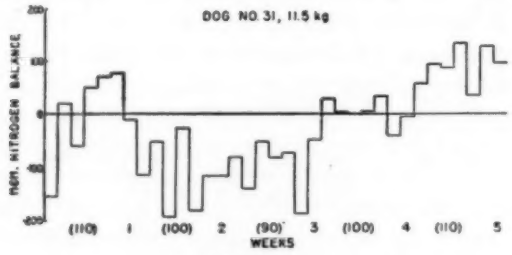

Figure 4

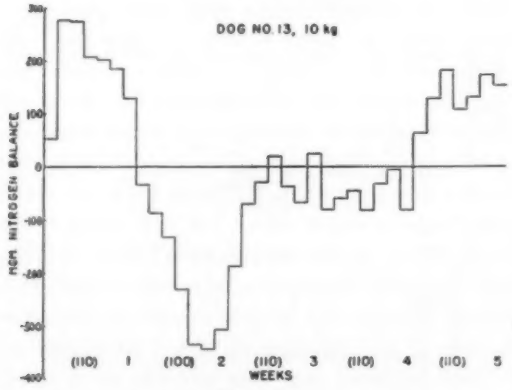

Figure 5

Figs. 4 and 5 Nitrogen balance with blood fibrin as the sole source of amino acids. The figures in parentheses indicate the daily nitrogen intake per kilogram of body weight during that week.

[2] Borden's lactalbumin 15–42, 12.6% nitrogen.

[3] Wilson Laboratories blood fibrin, 15.5% nitrogen.

gram of body weight, furnished as this blood fibrin, is sufficient for maintaining nitrogen balance in the adult dog.

Experiment 3. Casein.[4] The data in figures 6 and 7 show that approximately 130 to 140 mg of nitrogen per kilogram of body weight, furnished as this casein, is sufficient for maintaining nitrogen balance in the adult dog.

Experiment 4. Casein supplemented with adequate amounts of methionine.[5] The data in figures 8 and 9 show that approxi-

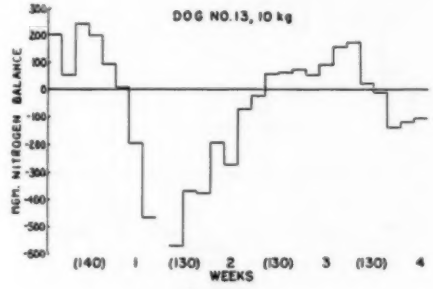

Figure 6

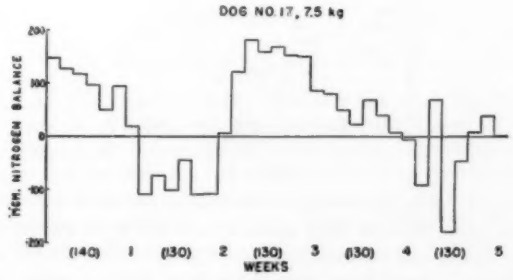

Figure 7

Figs. 6 and 7 Nitrogen balance with casein as the sole source of amino acids. The figures in parentheses indicate the daily nitrogen intake per kilogram of body weight during that week. During the third week 20 mg of additional methionine was added per day.

[4] Sheffield new process casein, 14.14% nitrogen.

[5] Sufficient methionine added to bring total daily methionine intake to 53 mg per kilogram of body weight. Casein was assumed to have 3.2% methionine (Block and Bolling, '45).

mately 90 mg of nitrogen per kilogram of body weight, furnished as casein supplemented with adequate amounts of methionine, is sufficient for maintaining nitrogen balance in the adult dog.

Experiment 5. Hydrolysates of casein. The data of figures 10, 11, 12, 13 and 14 show that approximately 130 to 140 mg of

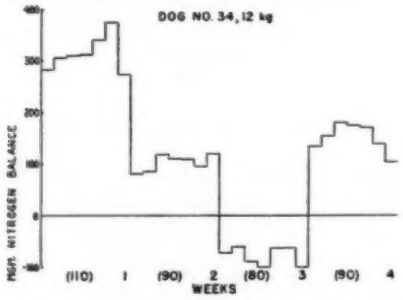

Figure 8

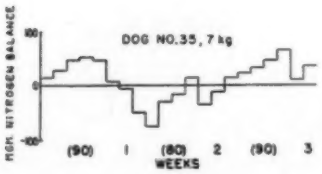

Figure 9

Figs. 8 and 9 Nitrogen balance with casein supplemented with sufficient methionine to bring the total daily methionine intake to 53 mg per kilogram of body weight. Casein was assumed to have 3.2% methionine (Block and Bolling, '45). The figures in parentheses indicate the daily nitrogen intake per kilogram of body weight during that week.

nitrogen per kilogram of body weight, furnished as hydrolysates of casein prepared by enzymatic hydrolysis, partial acid hydrolysis or complete acid hydrolysis and fortified with tryptophane, is sufficient for maintaining nitrogen balance in the adult dog.

Experiment 6. An acid hydrolysate of casein [6] *supplemented with adequate amounts of tryptophane and methionine.* The data in figures 15, 16, 17 and 18 show that approximately 90 mg of nitrogen per kilogram of body weight, furnished as a partial acid hydrolysate of casein and supplemented with adequate

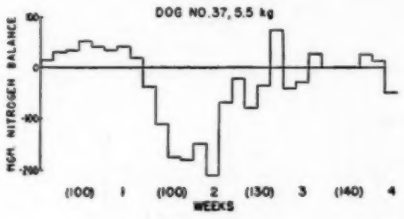

Figure 10

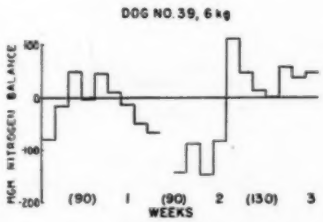

Figure 11

Figs. 10 and 11 Nitrogen balance with a partial acid hydrolysate of casein (Sahyun, Kade and Houston, '47) supplemented daily during the first week with 20 mg per kilogram of body weight of DL-tryptophane and 35 mg per kilogram of body weight of DL-methionine, and supplemented daily during the second, third and fourth weeks with 20 mg per kilogram of body weight of DL-tryptophane. The figures in parentheses indicate the daily nitrogen intake per kilogram of body weight during that week.

quantities of methionine and tryptophane, will maintain nitrogen balance in the adult dog.

Figure 19 summarizes briefly the minimum nitrogen requirements of the adult dog for the maintenance of nitrogen balance when the nitrogen is furnished by the tested proteins or hydrolysates.

[6] Sheffield new process casein, 14.14% nitrogen.

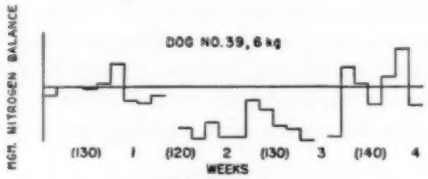

Figure 12

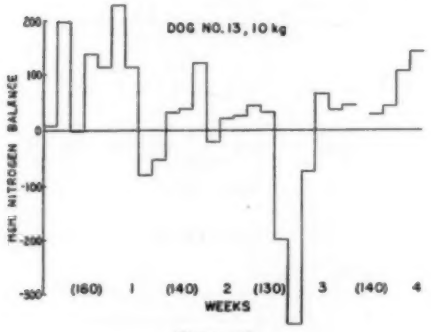

Figure 13

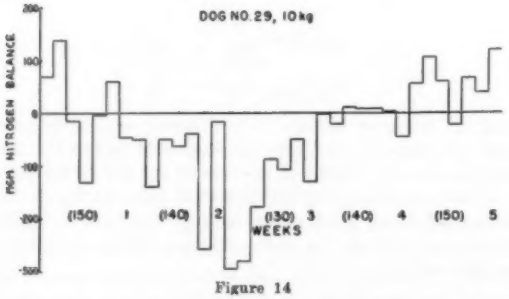

Figure 14

Fig. 12 Nitrogen balance with a partial acid hydrolysate of casein (Sahyun, Kade and Houston, '47) supplemented daily with 20 mg per kilogram of body weight of DL-tryptophane. The figures in parentheses indicate the daily nitrogen intake per kilogram of body weight during that week.

Fig. 13 Nitrogen balance with a complete acid hydrolysate of casein (Sahyun, '41) supplemented daily with 20 mg per kilogram of body weight of DL-tryptophane. The figures in parentheses indicate the daily nitrogen intake per kilogram of body weight during that week.

Fig. 14 Nitrogen balance with an enzymatic digest of casein (B-C-4-13) as the sole source of amino acids. The figures in parentheses indicate the daily nitrogen intake per kilogram of body weight during that week.

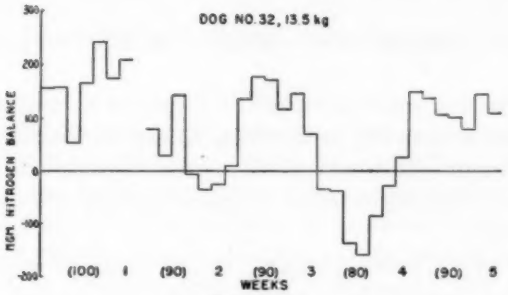

Figure 15

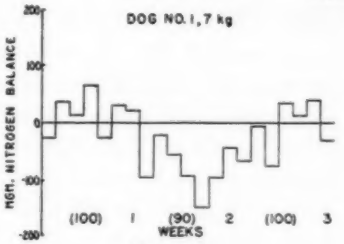

Figure 16

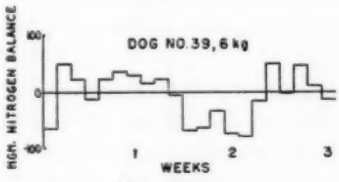

Figure 17

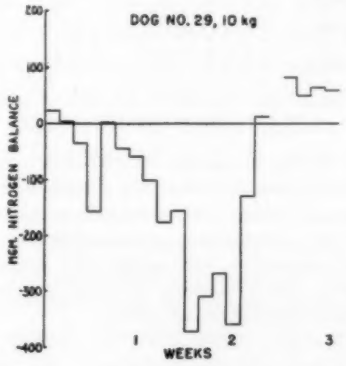

Figure 18

Figs. 15 and 16 Nitrogen balance with a partial acid hydrolysate of casein (Sahyun, Kade and Houston, '47) supplemented daily with 20 mg per kilogram of body weight of DL-tryptophane and sufficient DL-methionine to bring the total daily methionine intake to 53 mg per kilogram of body weight. The hydrolysate was assumed to have 3.2% methionine. The figures in parentheses indicate the daily nitrogen intake per kilogram of body weight during that week.

Figs. 17 and 18 Nitrogen balance with a partial acid hydrolysate of casein fed at a level of 90 mg per kilogram of body weight per day and supplemented daily during the first and third weeks with 20 mg per kilogram of body weight of DL-tryptophane and sufficient DL-methionine to bring the total daily methionine intake to 53 mg per kilogram of body weight. The hydrolysate was assumed to have 3.2% methionine. During the second week the methionine was omitted.

Considerably more nitrogen is required to maintain equilibrium when it is fed in the form of casein than in the form of lactalbumin. However, if the casein is supplemented with methionine, the requirement is approximately the same as for lactalbumin.

Casein hydrolysates prepared by enzymatic or acid degradation, either completely or only partially hydrolyzed, are equal in their ability to maintain nitrogen equilibrium in the adult

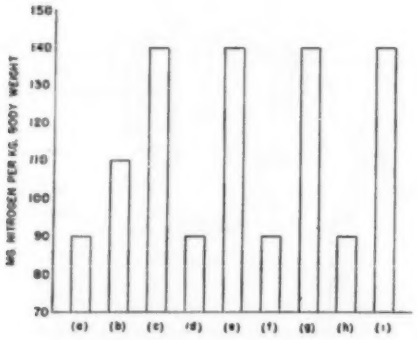

Fig. 19 Minimum quantity of nitrogen, furnished as protein or hydrolysates of protein, necessary for maintenance of nitrogen balance in the adult dog: (a) lactalbumin, (b) fibrin, (c) casein, (d) casein plus methionine, (e) complete acid hydrolysate of casein plus tryptophane, (f) complete acid hydrolysate of casein plus tryptophane and methionine, (g) partial acid hydrolysate of casein plus tryptophane, (h) partial acid hydrolysate of casein plus tryptophane and methionine, and (i) enzymatic hydrolysate of casein.

dog if the tryptophane destroyed in processing is restored. These hydrolysates are equivalent to the native casein in the maintenance of nitrogen balance. Approximately 140 mg of nitrogen per kilogram of body weight must be furnished, either as the native casein or as hydrolysates prepared from it. Adding adequate methionine to any of these hydrolysates of casein or to the casein itself lowers the nitrogen requirement to 90 mg per kilogram of body weight.

DISCUSSION

While this study was in process, a publication by Risser ('46) appeared in which much the same method was described. In general, the results reported here are in agreement with those of Risser. The discrepancies in the approximate values obtained for casein and fibrin can probably be ascribed to the differences in methods used for determining the standard weight of the animal and for calculating the amount of nitrogen fed. Risser used the kennel weight of the dog, but the experience in our laboratory is that the animals are always underweight when received and that after a few weeks on this diet they gain significantly.

In spite of adjusting the food intake to maintain a steady nutritive state, all animals on these diets continued to gain weight though more slowly in negative nitrogen balance. The rate of increase became very small after the dog reached a nutritive state of 0.30. No changes in weight over weekly periods as large as those reported by Risser were observed.

Since the animals obtained approximately 10 mg of nitrogen per kilogram of body weight from the basic diet, primarily as the vitamins, the actual nitrogen intake from the amino acid source was less by this amount than the total nitrogen intake indicated. However, since the balance is based on the difference between intake and excretion, and the non-amino acid nitrogen is constant and necessary, all results are expressed on the basis of total nitrogen required to maintain balance. If this 10 mg of nitrogen per kilogram of body weight were subtracted from values reported here, there would be no significant differences between these results for casein and fibrin and those reported by Risser.

Risser ('46) suggests that the difference in the utilization of casein and fibrin might be due to "incomplete hydrolysis of the protein in the digestive tract." In this connection it is interesting to note that a complete hydrolysate, the partial acid hydrolysate, or an enzymatic hydrolysate of casein is no better than the casein itself. This tends to eliminate the possibility that the sulfur-containing amino acids in casein are

not liberated at a sufficiently rapid rate to be fully utilized. It would be a most unusual coincidence if the destruction of the sulfur-containing amino acids and removal in the preparation of these different hydrolysates should be exactly equal to "the slower enzymatic release." Moreover, the fact that the addition of the methionine to casein and to its hydrolysate produces almost exactly the same effect seems to indicate that it is the pattern of amino acids rather than incomplete hydrolysis which accounts for the lower utilization of casein in comparison with fibrin or lactalbumin.

It is unlikely that strepogenin or other unidentified weight growth factors (Sprince and Woolley, '45; Rose and Womack, '46) play any role in the utilization of these proteins and hydrolysates in the adult animals since there probably are none present in the complete acid hydrolysate which by this test is equivalent to the native casein which does contain strepogenin.

It is important to note that all these minima are for adult animals. It is entirely possible that the minimum levels of these proteins or hydrolysates will not furnish an adequate amount of some one of the amino acids required by the growing animal. In addition, the growing animal's need for one or more amino acids in particular may be so much larger that the limiting amino acid in the utilization of the mixture may not be the same. Thus, the relative values of proteins may not be the same for growing as for adult animals.

Species differences in amino acid requirements are also very likely to affect both the quantitative requirement and the relative value of the protein or mixture. However, data obtained on 1 species under 1 set of conditions will necessarily have qualitative meaning for other species whose amino acid requirements are qualitatively similar.

SUMMARY

Since the biological value of any protein or mixture of amino acids will be dependent, at least in part, on the amino acid that is present in the lowest proportion to its quantitative re-

quirement, it is possible to determine the relative values of nitrogen sources by determining the minimum quantity necessary for maintenance of nitrogen balance. It has been shown that only 90 mg of nitrogen per kilogram of body weight is required in the form of lactalbumin, and 140 mg of nitrogen per kilogram of body weight as casein, a partial acid hydrolysate of casein fortified with tryptophane, a complete acid hydrolysate of casein fortified with tryptophane, or an enzymatic hydrolysate of casein. However, when fortified with adequate amounts of methionine, either casein itself or the hydrolysates will maintain nitrogen balance with only 90 mg of nitrogen per kilogram of body weight.

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THE ENERGY EXPENDITURE OF 9- TO 11-YEAR-OLD BOYS AND GIRLS (1) STANDING DRAWING AND (2) DRESSING AND UNDRESSING

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The need for more accurate information concerning the energy expenditure of boys and girls of all ages is felt whenever the problem of recommending Calorie allowances for children arises. Most of our present knowledge of the energy needs of boys and girls has come from studies of food consumption in relation to gains in height and weight. As noted by Taylor, Lamb, Robertson and MacLeod ('48), few measurements of the actual energy expenditure of children have been made as compared with the large number on adults. That children spend more energy per kilogram of body weight in certain activities than do adults engaged in the same activities has been found in studies conducted in this laboratory. Thompson ('40), comparing the energy expenditure of young girls during quiet play and cycling with that of college women engaged in the same activities under identical conditions, found the energy cost of quiet play (exclusive of the basal metabolism) for the girls to be more than 3 times that for the women and the difference for cycling was of about the same order of magnitude. Similar differences for boys and

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college men were found by Lamb ('42). Such comparisons emphasize the need for more accurate information concerning the energy requirements of children.

Since differences in the daily activities of children begin to be more varied at an age when physical development and muscle coördination are such that they can and do perform many of the same tasks as adults, the age range of 9 to 11 years was selected as the starting point for an extensive study of the energy expenditure of boys and girls in a number of activities. A survey was made of the activities engaged in by a large number of 9- to 11-year-old boys and girls living under different conditions.[2] As was anticipated, this survey revealed a great variety of activities which need to be investigated. It was decided to investigate first those which are common to most children. This paper reports the energy expenditure of 9- to 11-year-old boys and girls when standing drawing (an activity common to all school children who work at blackboards) and when dressing and undressing.

SUBJECTS

Seven boys and 12 girls, ranging in age from 9 to 11 years, were selected as experimental subjects. Some of these children attended a private school and lived at home with their parents, and some came from a children's home where they lived under more routine conditions. Health records reported by physicians were studied to ascertain the health status of the children and to make sure that they had no physical defects and might be considered average normal individuals. The weight, height, surface area and deviations from predicted weight for height and age (Baldwin-Wood standards) were determined. The average values are given in table 1.

PROCEDURE

The activity studies were carried out in the open-circuit respiration chamber described by Taylor, Lamb, Robertson

[2] Findings of this survey will be reported at another time.

and MacLeod ('48). The total weight of carbon dioxide produced during an experimental period was determined by analysis of a sample of the chamber air at the beginning and at the end of the period and adding the difference to the increase in weight of the absorbing train during the period. The Carpenter modification of the Haldane gas analysis apparatus ('23, '29) was used for determining the carbon dioxide content of the chamber air. The carbon dioxide produced by 2 children during an experimental period was divided by 2 to obtain the average carbon dioxide production per child.

Two walls of the chamber were covered with large sheets of drawing paper placed at blackboard height, and pictures, which could be copied or colored, were also placed on the walls to provide inspiration for those children whose imaginations needed a little stimulation. One wall of the respiration chamber was fitted with 2 small mirrors placed at suitable height so that the children could comb their hair without any undue stooping or stretching. The activity studies were conducted in the afternoon after school and the children were asked to bring gymnasium clothes for the dressing and undressing activity.

On arrival in the laboratory, the children placed their clothes in small suitcases and each child was given a box of colored crayons and a game or puzzles to take into the chamber. Two boys or 2 girls were studied at a time. In this way it was possible to keep the children in the respiration chamber for a shorter period of time and still collect sufficient carbon dioxide to maintain a satisfactory degree of accuracy. Also, for an experimental period lasting 35 minutes, it was more fun for the children when 2 of them could be subjects at the same time. Before entering the chamber they were given detailed instructions regarding the procedure for the test.

After entering the chamber they sat quietly for a 10-minute rest period during which time they were permitted to play Chinese checkers or to work on "Goofy" puzzles. They were then given the signal to start drawing and this activity was continued for a period of 15 minutes. They were free to draw

TABLE 1

Average weight, height, deviation from predicted weight, surface area and basal metabolism of subjects (figures in parentheses show range)

	WEIGHT		HEIGHT		DEVIATION FROM PREDICTED WEIGHT	SURFACE AREA	BASAL METABOLISM IN CALORIES PER			
							24 hr.	kg/hr.	m^2/hr.	cm of ht./ 24 hr.
	lb.	*kg*	*in.*	*cm*	%	m^2				
Boys										
7 subjects	77.2	35.1	55.1	139.9	+ 5.0	1.16	1192	1.42	42.8	8.5
Age range										
9-8 to 10-8 [1]	(69.3–	(31.5–	(52.3–	(132.8–	(— 5.4–	(1.06–	(1049–	(1.28–	(39.3–	(7.7–
	84.5)	38.4)	57.9)	147.0)	+ 16.2)	1.25)	1391)	1.57)	46.7)	9.6)
Girls										
12 subjects	73.9	33.6	55.1	139.9	— 1.1	1.15	1186	1.49	43.0	8.4
Age range										
8-10 to 11-5 [1]	(59.6–	(27.1–	(51.0–	(129.6–	(— 10.3–	(0.99–	(914–	(1.29–	(37.7–	(6.7–
	96.6)	43.9)	59.1)	150.1)	+ 11.0)	1.35)	1473)	1.69)	48.1)	9.8)

[1] First number in each set refers to years, the second to months.

anything they wished but were asked to keep actively engaged in standing drawing throughout the 15-minute experimental period. At the end of the drawing period they were given the signal to start undressing and dressing which was continued for 10 minutes. During this period they changed their clothes, getting into gymnasium clothes and back again into their regular school clothes, and changed their shoes and combed their hair. Most of the children carried out this routine more than once during the 10-minute period allotted to dressing and undressing.

Basal metabolism determinations were made on all subjects by means of the Benedict-Roth and the Sanborn-Benedict respiration apparatus, under generally accepted standard conditions. Two determinations were made on each child for as many mornings as were required to obtain the average of at least the 3 lowest figures checking within 5%. Usually 2 mornings sufficed. Basal metabolism determinations and activity studies were made on each child within a 6-month period. A summary of the basal metabolism determinations is given in table 1. In each case the basal metabolism of the children included in the study agreed within the commonly accepted normal range of ± 15% with 2 or more of the standards currently used for the basal metabolism of children (Dreyer, '20; Boothby, Berkson and Dunn, '36; Talbot, '38; Lewis, Duval and Iliff, '43).

RESULTS AND DISCUSSION

The average results of the activity studies are summarized in table 2. Eleven cases were obtained on boys and 14 cases on girls for each of the activities, and the results expressed in terms of total Calories per hour per kilogram of body weight and per centimeter of height.

Standing drawing

The average energy expenditure for standing drawing by the boys was found to be 3.19 ± 0.10 Cal. per kilogram of body weight per hour and 0.83 ± 0.03 Cal. per centimeter of

height per hour, and by the girls, 2.62 ± 0.05 Cal. per kilogram of body weight per hour and 0.63 ± 0.01 Cal. per centimeter of height per hour. That the boys spent more energy than the girls in performing the same activity is possibly accounted for by the fact that in general the boys in drawing tended to

TABLE 2

Average energy expenditure of children (1) standing drawing and (2) dressing and undressing (including the basal metabolism)

	STANDING DRAWING		DRESSING AND UNDRESSING	
	Boys	Girls	Boys	Girls
CO_2 produced/ child/hr. (gm)				
Mean ± P.E.[1]	38.39 ± 1.69	29.19 ± .64	50.35 ± 1.97	46.47 ± 1.35
No. of cases	11	14	11	14
C.V.[2] (%)	21.7	12.1	19.3	16.1
P.E. as per cent of mean	4.4	2.2	3.9	2.9
Cal./kg/hr.				
Mean ± P.E.	3.19 ± .10	2.62 ± .05	4.29 ± .16	4.04 ± .13
No. of cases	11	14	11	14
C.V. (%)	15.8	9.6	18.9	18.1
P.E. as per cent of mean	3.1	1.9	3.7	3.2
Cal./cm ht./hr.				
Mean ± P.E.	0.83 ± .03	0.63 ± .01	1.09 ± .04	1.00 ± .03
No. of cases	11	14	11	14
C.V. (%)	20.5	9.5	18.3	14.9
P.E. as per cent of mean	3.6	1.6	3.7	3.0

[1] Probable error.
[2] Coefficient of variation.

work with more flourish than the girls. Their sketches were carried out on a large scale and they usually covered all available space provided for drawing, their pictures showing considerable initiative. On the contrary, the girls tended to confine their drawing to a much smaller space and there was a

stronger tendency simply to color the outline drawings provided than to make original sketches of their own. When given the opportunity to draw under identical conditions this difference between the boys and girls was found whether they came from the home for children or the private school. The difference is seen in the higher coefficients of variation for the boys, 15.8 and 20.5, respectively, as compared with 9.6 and 9.5 for the girls.

Compared with the basal metabolism, there is an increase in energy expenditure per kilogram per hour for standing drawing of 125% for the boys and 76% for the girls. There was no significant difference in the energy expenditure for this activity between the subjects coming from the home for children and those coming from the private school. The energy expenditure for standing drawing may be taken as fairly typical of that for boys and girls doing problems or drawing on the blackboard, or making murals, an activity quite common in progressive schools throughout the country.

Dressing and undressing

The average energy expenditure for dressing and undressing by the boys was found to be 4.29 $\pm$ 0.16 Cal. per kilogram of weight per hour and 1.09 $\pm$ 0.04 Cal. per centimeter of height per hour, and by the girls, 4.04 $\pm$ 0.13 Cal. per kilogram of weight per hour and 1.00 $\pm$ 0.03 Cal. per centimeter of height per hour. These figures indicate less difference between boys and girls in the energy they expend for dressing and undressing than for standing drawing. The coefficients of variation are 18.9% per kilogram of weight and 18.3% per centimeter of height for the boys, and 18.1% per kilogram of weight and 14.9% per centimeter of height for the girls. That coefficients of variation are higher for both boys and girls when dressing and undressing than when standing drawing is to be expected since the motions involved in dressing and undressing are so much more variable. For example, sometimes both boys and girls spent more time in combing their hair and the boys more in fixing their neckties than they did

at other times. The increase in energy expenditure per kilogram per hour for dressing and undressing over the basal energy expenditure amounts to 202% and 171% for boys and girls, respectively.

SUMMARY

1. The energy expenditure of 7 boys and 12 girls, 9 to 11 years of age, selected from a home for children and from a private school, has been measured while they were standing drawing and while they were dressing and undressing in a respiration chamber.

2. The average energy expenditure by the boys while standing drawing was found to be 3.19 $\pm$ 0.10 Cal. per kilogram of weight per hour with a coefficient of variation of 15.8% and 0.83 $\pm$ 0.03 Cal. per centimeter of height per hour with a coefficient of variation of 20.5%. The average energy expenditure by the girls while standing drawing was found to be 2.62 $\pm$ 0.05 Cal. per kilogram of weight per hour with a coefficient of variation of 9.6% and 0.63 $\pm$ 0.01 Cal. per centimeter of height per hour with a coefficient of variation of 9.5%.

3. The average energy expenditure by the boys while dressing and undressing was found to be 4.29 $\pm$ 0.16 Cal. per kilogram of weight per hour with a coefficient of variation of 18.9% and 1.09 $\pm$ 0.04 Cal. per centimeter of height per hour with a coefficient of variation of 18.3%. The average energy expenditure by the girls while dressing and undressing was found to be 4.04 $\pm$ 0.13 Cal. per kilogram of weight per hour with a coefficient of variation of 18.1% and 1.00 $\pm$ 0.03 Cal. per centimeter of height per hour with a coefficient of variation of 14.9%.

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THE BIOLOGICAL VALUE OF A MEAT HYDROLYSATE IN THE INFANT

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The practice of administering protein digests to infants suffering from diseases which cause excessive nitrogen loss seemed to us to make it imperative that the biological value of preparations derived from various proteins be assessed in the normal infant prior to their therapeutic application. Accordingly, in a previous publication, we (Albanese and associates, '47) reported that the nutritional values of an enzymatic digest of casein [1] and lactalbumin [2] were equal to that of a diluted evaporated milk formula and superior to a synthetic diet in which a tryptophane and cystine supplemented acid digest of casein constituted the principal source of nitrogen. In this report the nutritive characteristics of a synthetic milk containing an enzymatic digest of beef muscle as the protein component are compared with those of the evaporated milk formula.

EXPERIMENTAL

Diets and procedures

The composition and caloric distribution of the 2 formulae employed in this study are shown in table 1. These were given in 5 feedings daily at the rate of 100 cal. and 0.56 gm of

[1] Amigen.
[2] Edamin.

nitrogen per kilogram of body weight and were supplemented daily with 50 mg of ascorbic acid and 15 drops of oleum percomorphum. Owing to uncertainties regarding the B complex vitamins required by the infant, brewers' yeast was used instead of a mixture of the synthetically available components of this vitamin complex. The amino acids derived from this source can be readily ascertained from the literature (Carter and Phillips, '44). The nitrogen content of each diet preparation was determined by the micro-Kjeldahl

TABLE 1

Composition and caloric distribution of diets

Evaporated milk formula (EM diet):			
Evaporated milk, 50 ml (70 cal.); corn sirup 10 ml (30 cal.); water 40 ml. Total volume 100 ml; total calories 100.			
Meat protein digest formula (MH diet):			
Meat digest (Wilson's,[1] 0.56 × 6.25)		3.5 gm	14.0 cal.
Mead Johnson Lab. Prod. no. 217		18.0 gm	
Fats	4.0 gm		36.0 cal.
Carbohydrates	11.6 gm		46.5 cal.
Mead Johnson Brewers' Yeast		1.0 gm	3.5 cal.
Water		88.0 ml	
Total		100.0 ml	100.0 cal.

Salt supplement mg per 100 ml: $FeSO_4$, 10; NaCl, 100; Ca gluconate, 790; KH_2PO_4, 323; $Ca(OH)_2$, 197; K_2HPO_4, 108; KCl, 90; MgO, 18.

[1] We are indebted to Dr. C. E. Graham, of the Wilson Laboratories, for a supply of this preparation.

method and the daily N intake of the subjects calculated from the consumption record. The diets were tested in 5 normal male infants (2.5–8 months of age) for 4 consecutive 7-day periods. Collection of the excreta was omitted on week-ends to avoid complications which might arise from constant use of restraints. Twenty-four hour urine specimens were collected by means of adapters in bottles containing 10 ml or 15% (by volume) HCl and 1 ml of 10% alcoholic thymol. The feces were collected in 19 cm porcelain evaporating dishes held in place by

a fitted excavation in the mattress and subsequently accumulated under refrigeration for each diet period in jars containing 200 ml of 70% alcohol. The infants were weighed and submitted to complete clinical examinations daily during the course of the study.

Data on the nitrogen retention were obtained by subtracting from the daily N intake the total N output as found by micro-Kjeldahl analyses of the daily 24-hour urine collections and period pools of the feces.

The effect of the dietary changes on the blood proteins was ascertained by analyses of blood samples (5 ml) collected on the last day of each diet period. The hemoglobin content of these specimens was determined colorimetrically by the acid hematin method in the Klett-Summerson photoelectric colorimeter. The total plasma proteins, albumin/globulin ratios and non-protein N were measured by the techniques previously described by us.

Results

The nitrogen retention and body weight gains induced by the evaporated milk formula and meat hydrolysate preparation indicate that by these criteria the 2 diets are of equal nutritional quality (table 2). Moreover, the observed nitrogen retention values fall well within the range of those previously reported by ourselves and other workers using a variety of milk formulae (Albanese and associates, '47).

Attention is called to the observation that a shift in the N intake of subject A.H. from 0.7 to 0.6 gm per kilo on either diet had no appreciable effect on the nitrogen retention and weight gain. This suggests that effects of hyperalimentation reported by Nelson ('30) become apparent only at lower nitrogen intake levels than were imposed in this instance.

The data on the total plasma protein levels given in table 2 furnish further evidence of the comparable biological value of the diets. No significant changes in the albumin-globulin ratios and hemoglobin concentration of the blood were observed.

The amounts of the essential and some non-essential amino acids provided per kilo of body weight by the meat hydrolysate diet were computed from our analyses of the meat digest and the available amino acid data on the brewers' yeast (Carter and Phillips, '44). These results and corresponding figures

TABLE 2

Biological value of evaporated milk and meat hydrolysate formulae in the normal infant

SUBJECT	DIET	TOTAL N INPUT	DAILY BODY WEIGHT CHANGE	NITROGEN RETENTION	TOTAL PLASMA PROTEINS
		gm	*gm*	*mg/kg*	*gm %*
J.A.,	EM	2.58	+ 14	122	6.18
2.5 months,	MH	2.36	+ 21	139	6.06
4,218 gm	MH	2.36	+ 20	116	5.75
	EM	2.53	+ 21	177	6.15
R.M.,	EM	3.03	+ 21	118	5.29
4.0 months,	MH	2.73	+ 28	107	5.30
4,871 gm	MH	2.73	+ 25	102	5.12
	EM	2.84	+ 24	106	5.36
A.H.,	EM	5.22	+ 14	194	6.41
7 months,	MH	4.22	+ 26	187	6.60
7,154 gm	MH	5.22	+ 21	205	6.50
	EM	4.66	+ 25	185	6.35
D.C.,	EM	3.86	+ 28	150	6.06
8 months,	MH	3.83	+ 14	155	6.55
5,309 gm	MH	3.86	+ 25	140	5.68
	EM	4.10	+ 18	140	6.04
W.G.,	EM	4.02	+ 14	161	5.82
8 months,	MH	4.21	+ 20	156	5.30
6,683 gm	MH	4.02	+ 21	157	5.62
	EM	3.97	+ 17	144	6.25

for the evaporated milk diet which were derived from Williamson's ('44) analyses of cow's milk are given in table 3. Examination of the values listed under the column heading (b)-(a) reveals that the meat hydrolysate diet furnishes more of all the essential amino acids except phenylalanine, isoleucine and

leucine. The low isoleucine content of the meat hydrolysate diet would not seem to constitute a limiting factor since the 102 mg of isoleucine provided by this diet is well above the 90 mg of isoleucine per kilo of body weight found by Albanese and coworkers ('48) to be required by the infant. Inasmuch as the 2 diets were found to be of equal biological value, it can be inferred that the phenylalanine and leucine deficits of the meat hydrolysate diet are negligible or are compensated

TABLE 3

Amino acid intake of infants on the evaporated milk and meat hydrolysate diets (all values are given in mg per kilo of body weight)

AMINO ACID	EVAPORATED MILK	MEAT HYDROLYSATE FORMULA			
	(a)	Meat hydrolysate	Brewers' yeast	Total (b)	(b)−(a)
	mg	*mg*	*mg*	*mg*	*mg*
Arginine	127	185	21	206	+ 79
Cystine	27	40	7	47	+ 20
Histidine	63	115	19	134	+ 71
Isoleucine	167	86	16	102	— 65
Leucine	490	360	65	425	— 65
Lysine	200	288	32	320	+ 120
Methionine	99	104	13	117	+ 18
Phenylalanine	177	149	20	169	— 8
Threonine	151	209	25	234	+ 183
Tryptophane	47	87	6	93	+ 46
Tyrosine	172	91	21	112	— 60
Valine	171	205	22	227	+ 56

in part by the excess of the other essential amino acids. A comparison of the data secured in this study with those previously reported (Albanese and associates, '47) for the enzymatic digests of casein and lactalbumin suggests that the biological value of the hydrolysate employed in this investigation is equal to that of the milk protein preparations.

SUMMARY

It has been found that the nitrogen retention and weight gain of infants maintained on a synthetic milk, in which an enzymatic digest of beef muscle constituted the principal

source of nitrogen, were equal to those obtained on an evaporated milk formula fed at the same fluid, caloric distribution and nitrogen levels.

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THE RELATION BETWEEN STARVATION, METABOLIC ACIDOSIS AND CONVULSIVE SEIZURES IN RATS[1]

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TWO FIGURES

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In the course of an investigation of the metabolic determinants of convulsive seizures in pyridoxine-deficient rats it was observed that pair-fed control rats receiving a qualitatively adequate but quantitatively inadequate diet had lower electroshock thresholds than comparable animals allowed to eat at will. Further investigation showed that the susceptibility of rats to electrically induced convulsions could be increased by restricting the caloric intake and rapidly decreased by refeeding. This result was not anticipated, for Geyelin ('21) claimed that fasting reduces the incidence of seizures in epileptic patients. The benefit of fasting has been attributed to the attendant metabolic acidosis, and Wilder ('21), Peterman ('24) and others have asserted that a ketogenic diet which produces metabolic acidosis reduces the incidence of seizures in epileptic children. (See Lennox and Cobb ['28] for a thorough review of the older clinical literature.) Because our preliminary laboratory observations did not seem to agree with clinical experience, the relation between starvation, metabolic acidosis and brain excitability in rats was reëxamined with the results described below.

[1] This work was supported by the United States Public Health Service Research Grant 155-c for the Study of the Physiology and Therapy of Convulsive Disorders.

METHODS

Male rats of the Sprague-Dawley strain were used. Precautions were taken to insure an uninterrupted supply of water and relative constancy of room temperature, factors which in our experience have an important effect upon the electroshock threshold. Two stock diets were used. Diet A consisted of a commercial dog food [2] plus 15 gm of green vegetables per rat 3 times a week. Diet B consisted of a commercial calf meal [3] with 3% brewers' yeast and 3% wheat germ added.

The electroshock thresholds were determined with a specially constructed constant-current apparatus utilizing Spiegel corneal electrodes. The duration of current was 0.2 seconds, and the intensity was increased by increments of 1.0 milliampere or less at intervals of 1 hour until a minimal clonic seizure occurred. Under our experimental conditions, the mean threshold of any group of normal rats was almost perfectly constant from day to day or over a period of several days.

In examining maximal seizure patterns, the same apparatus was used and the same duration of exposure, but the intensity of the current was 150 milliamperes.

Blood was obtained for acid-base studies by heart puncture in unanesthetized rats. Blood was drawn into a 2 ml syringe containing a drop of 1% heparin solution, and it was handled anaerobically by the method of Davenport ('47a). The plasma pH was measured with a glass electrode, and the total carbon dioxide content of the plasma was determined by the method of Van Slyke and Neill ('24). The bicarbonate concentration of the plasma in millimoles per liter was calculated using pK of 6.10 and carbon dioxide solubility coefficient of 0.0301.

Respiratory acidosis or alkalosis may be present at the time the blood samples are taken, because asphyxia may be induced by holding the rats for heart puncture, or hyper-

[2] Purina Dog Chow.

[3] Known as "Calf Builder" and prepared by General Mills, Inc., Minneapolis, Minn.

ventilation may accompany their struggles. Nevertheless, the presence and extent of metabolic acidosis can be estimated by a valid graphical method. Blood samples from 10 normal rats having no respiratory acidosis or alkalosis were found to have an average pH of 7.41 and a plasma bicarbonate concentration of 24.3 mM/l. This was plotted as the normal point on a pH-bicarbonate diagram in which ordinates were plasma bicarbonate concentrations in mM/l and abscissae were pH units. The normal buffer curve of rat blood was found by determining the carbon dioxide absorption curve of rat blood, and when plotted on the pH-bicarbonate diagram it was found to be a straight line having a slope of −24.7 mM/l/pH unit. A line having such a slope was drawn through the normal point.

When the plasma bicarbonate and pH of any blood sample are plotted as a point on the diagram the vertical distance between the point and the buffer line is a measure of the extra fixed acid present in the blood in mM/l. The pH of the blood, if no respiratory acidosis or alkalosis is present can be estimated by drawing a line through the point parallel to the normal buffer line and reading the pH at which this line crosses the isobar representing a pCO_2 of 40 mm Hg. A complete description of the theoretical basis of this graphical method has been given by Davenport ('47b).

RESULTS

Starvation and electroshock threshold

Ten young rats having an average body weight of 124 gm were placed on diet A and allowed to eat at will. Their food consumption, which averaged about 15 gm per rat, was measured daily, and their body weights and electroshock thresholds were determined at intervals of several days. The mean body weights and electroshock thresholds over a period of 121 days were recorded as the solid lines in figure 1.

A second group of 10 young rats having an average body weight of 127 gm was placed on diet A, and in the first 85

days the rats were given 4 to 6 gm of food each per day, the amount given being chosen in order to keep their body weights constant. After the 85th day they were allowed to eat at will. Their mean body weights and electroshock thresholds

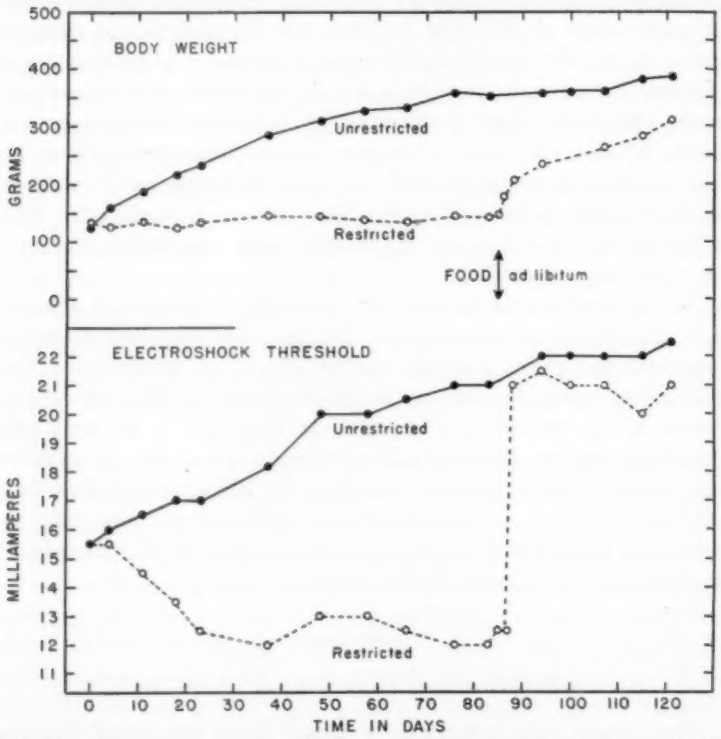

Fig. 1 Upper curves: average body weight of rats fed unrestricted (solid lines) and restricted (broken lines) amounts of diet A. Lower curves: average electroshock thresholds in milliamperes of the same rats.

over a period of 121 days are recorded as the broken lines in figure 1.

The electroshock thresholds of the rats on unrestricted food intake increased *pari passu* with the body weights. The body weights of the rats on restricted food intake remained

constant. Their electroshock thresholds fell during the first 23 days and thereafter remained significantly below the original thresholds. After the animals were allowed to eat at will their body weights began to increase slowly, but within 24 hours their electroshock thresholds rose abruptly to the level of the first group and remained high throughout the rest of the experiment.

The experiment was repeated twice with groups of rats of higher and lower initial weights, and the same results were obtained.

It is possible to raise or lower the electroshock threshold by varying the nutritional state of the rat as shown in figure 2. Ten rats whose average daily consumption of diet B was 15.5 gm each were given 5 or 6 gm per rat for 36 days. On the 37th and 38th days they were allowed to eat at will. Their food intake was again restricted until the 52nd and 53rd days when they were once more allowed to eat at will. During the initial period of food restriction the average electroshock threshold fell from 20.0 mA to 16.5 mA. Within 48 hours of the beginning of each period of unrestricted food intake the electroshock thresholds rose abruptly to the initial level and then fell slowly during the subsequent period of restriction.

Starvation and seizure pattern

The pattern of maximal seizures as well as the electroshock threshold is changed by starvation. Toman, Swinyard and Goodman ('46) have shown that the normal seizure pattern in rats is typical, consisting of a latent period, a period of tonic flexion, a period of tonic extension, a clonic phase which is frequently absent, and a period of post-seizure depression. The time from the application of the shock to the beginning of the extensor phase is 2.8 seconds and the time from the application of the shock to the end of the extensor phase is 14 seconds.

Rats having reduced electroshock thresholds as the result of starvation show the same sequence in maximal seizures as

do normal rats, but the time relationships are altered. The latent period is so short as to be almost undiscernible; the time to the beginning of the extensor phase is about 1.5 seconds; and the time to the end of the extensor phase is

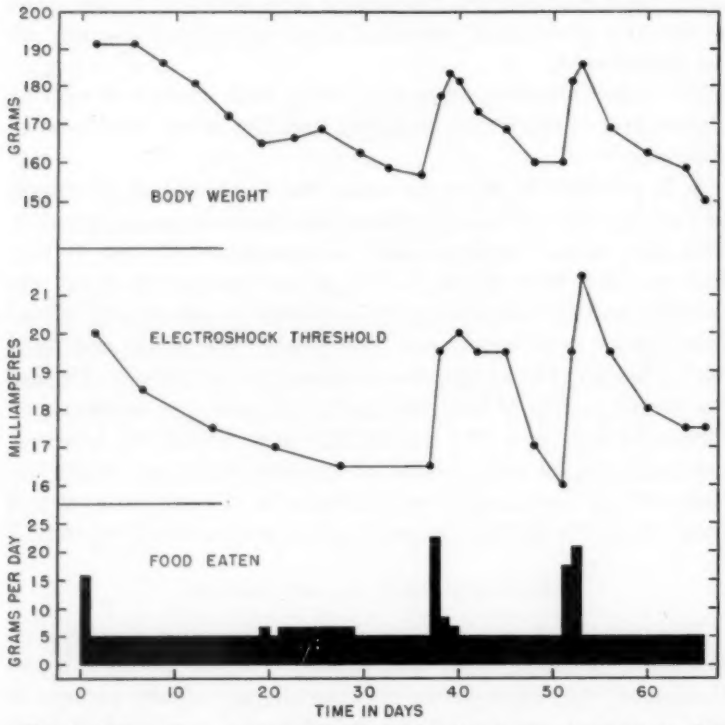

Fig. 2 Bottom bars: average amount of food eaten in grams per rat per day. Middle curve: average electroshock threshold in milliamperes. Upper curve: average body weight in grams.

lengthened to as much as 30 or 35 seconds. Unlike the electroshock threshold, the seizure pattern does not return entirely to normal within 48 hours after the animals are allowed access to unrestricted amount of food. A week or more of refeeding is required before the time relationships become normal.

Effect of dietary supplements

In order to determine which dietary component raises the electroshock threshold, control rats and rats on a restricted diet were allowed to eat the supplements listed in table 1. The control rats were given diet B in unrestricted amounts, and rats in another group were each given 5 gm daily of the same diet for 6 to 7 weeks before the supplements were fed. Both groups were provided with the supplements, with the exception of the vitamin mixture and the ammonium chloride, in unrestricted amounts for 2 days, and the amounts consumed were recorded. The electroshock thresholds were determined immediately before and 2 days after the beginning of the supplement.

The vitamin mixture was brought into aqueous solution by neutralization with sodium bicarbonate, and was given to the animals in 2 daily subcutaneous injections for 2 days. Each rat received the following amounts in milligrams daily: thiamine, pyridoxine, riboflavin and nicotinamide 1.0 each; calcium pantothenate 5.0; inositol 10.0; p-aminobenzoic acid 20.0; choline hydrochloride 20.0 and 2-methyl-naphthoquinone 0.1. The agar supplement was moistened purified bacteriological agar. The carbohydrate was commercial cornstarch, which

TABLE 1

Effect of dietary supplements on electroshock threshold.

SUPPLEMENT	UNRESTRICTED					RESTRICTED				
	No. of rats	Amount eaten	Electroshock threshold			No. of rats	Amount eaten	Electroshock threshold		
			Initial mA	Final mA	p [1]			Initial mA	Final mA	p [1]
		gm					*gm*			
Vitamins	5	..	21.8	22.0	0.4	10	..	17.1	17.2	0.6
Agar	20	5.2	23.9	23.8	0.7	20	9.2	17.2	17.3	0.9
Carbohydrate	10	35.8	23.3	23.5	0.5	10	31.0	17.1	20.5	0.01
Protein	10	15.8	23.0	23.0	0.9	19	19.0	16.8	19.7	0.01
Fat	20	15.8	22.9	21.0	0.01	21	19.2	17.9	19.2	0.01
Fat + 1% choline	10	16.2	24.2	22.4	0.01	10	17.7	16.7	18.9	0.01
NH_4Cl	12	0.5 mM/100 gm	19.0	19.0	0.9					
NH_4Cl	18	2.0 mM/100 gm	19.8	22.5	0.01					

[1] Fisher, '36.

was preferred to sucrose because it did not produce diarrhea and loss of electrolytes, with consequent alteration of the electroshock threshold. Protein was given as commercial vitamin-free casein. The fat supplement was hydrogenated cottonseed oil [4] or this material plus 1% choline hydrochloride. Ammonium chloride was given by stomach tube in a dose of 2.0 or 0.5 mM per 100 gm of rat. In this instance thresholds were determined 2 hours after the administration of the ammonium chloride.

The results are recorded in table 1. The initial and final thresholds of each rat were compared, and the significance of the difference, given in the fifth and last columns, was calculated by Fisher's ('36) method for determining the significance of the means of a unique sample.

There was no significant change in electroshock threshold in any group of rats receiving vitamins or agar. There was no change in the normal rats receiving carbohydrate or protein, but there was a significant fall in threshold in the normal rats receiving fat or fat plus 1% choline. There was a significant rise in threshold of rats on the restricted diet receiving carbohydrate, protein, fat or fat plus 1% choline. There was a significant rise in threshold of rats receiving 2.0 mM/100 gm ammonium chloride, but no change in the rats receiving 0.5 mM/100 gm.

Metabolic acidosis

The results of the acid-base analyses of the blood are given in table 2. Starvation and the feeding of fat or fat plus 1% choline resulted, as would be expected, in a mild metabolic acidosis. The urine of all the fat-fed rats gave an intensely positive reaction for ketone bodies. The administration of 0.5 mM/100 gm ammonium chloride produced a comparable mild metabolic acidosis whereas the administration of 2.0 mM/100 gm produced extreme metabolic acidosis.

[4] Crisco.

TABLE 2

Metabolic acidosis.

SUPPLEMENT	UNRESTRICTED			RESTRICTED		
	No. of rats	pH Range	Fixed acid mM/1	No. of rats	pH Range	Fixed acid mM/1
None	10	7.40–7.44	0	10	7.33–7.42	4.1 ± 7.3
Fat	10	7.16–7.37	7.1 ± 1.3 [1]	9	7.26–7.46	4.4 ± 1.1
Fat + 1% choline	10	7.25–7.43	4.8 ± 1.3	10	7.24–7.41	3.5 ± 1.0
NH_4Cl 0.5 mM/100 gm	10	7.25–7.40	6.0 ± 0.9			
NH_4Cl 2.0 mM/100 gm	10	6.81–7.06	23.9 ± 2.0			

[1] Standard error of the mean.

DISCUSSION

In rats allowed to eat at will the electroshock threshold rises as the body weight increases. As the rats grow, there is an increase in skull size and brain volume which doubtless contributes to the increase in electroshock threshold. It is remarkable, however, that the thresholds of rats which have been severely stunted by undernutrition rise at once, upon refeeding, to the threshold levels of control animals which have grown considerably since the beginning of the experiment. This fact suggests that not only increase in size but also other changes associated with increasing age determine the electroshock threshold. The results obtained on the rats with restricted caloric intake clearly show that, in addition, the nutritional state of the animals profoundly affects the electroshock threshold, for the threshold can be lowered by restricting the intake and abruptly raised by removing the restriction. In these circumstances there is no change in skull size and brain volume, and the changes in threshold must be the direct result of the changes in the nutritional state of the animals.

The results obtained with carbohydrate, fat and protein supplements show that the threshold of rats on a restricted intake can be raised by any one of the 3 major foodstuffs.

The means by which these diverse substances raise the threshold is at present unknown. It is possible that a simple reduction of the calories available disturbs brain excitability and that restoration of a normal supply of calories allows a return to the normal state; but it is equally possible that the changes are caused by a much more devious mechanism. Further analysis of the phenomenon may reveal much about the relation between the brain's metabolism and its function.

The reduction of electroshock threshold in normal rats fed large amounts of fat cannot be explained without further investigation. These animals lost weight during the period of fat feeding, and their feces contained large quantities of fat. It is possible that the 2-day period of fat feeding actually reduced their caloric intake temporarily, but it is unlikely that so brief an interval of reduced intake would affect the electroshock thresholds.

Patton and his collaborators ('41) have shown that rats maintained on a diet which is qualitatively adequate but quantitatively inadequate have an increased incidence of audiogenic seizures. The fact that the susceptibility of rats to electrically induced seizures is similarly influenced by chronic undernutrition suggests that the 2 types of seizure may have a common basis. On the other hand, the same investigators (Patton et al., '42) found that the incidence of audiogenic seizures resulting from chronic underfeeding in rats could be reduced by the administration of a mixture of vitamins and minerals. This does not agree with our observation that massive doses of the vitamins of the B-complex have no effect on the electroshock threshold. One explanation for the discrepancy lies in the fact that our animals were treated with the supplement for only 2 days, whereas no effect was noticed on the incidence of audiogenic seizures until after 5 days except in rats which had previously received inadequate amounts of thiamine. More probably, our diet B contained such large portions of the vitamins of the B-complex that the animals were already receiving optimal amounts.

The extreme metabolic acidosis produced by the administration of 2.0 mM/100 gm ammonium chloride causes a significant rise in threshold, but it is unlikely that the less severe metabolic acidosis observed in the other instances is responsible for any change in excitability. Metabolic acidosis was present in the starved animals having lowered thresholds, but a similar degree of metabolic acidosis was also present in the starved animals when the threshold was raised by fat feeding, and in the normal animals when it was lowered by the same diet. Finally, ammonium chloride in a dose sufficient to produce a comparable metabolic acidosis is without effect on the threshold. It can be concluded that mild metabolic acidosis does not in itself change the threshold.

The results presented in this paper have 3 important practical bearings. The first is that the design and interpretation of experiments on convulsive phenomena must always take into account the nutritional state of the animals used. Any experimental procedure such as vitamin deprivation or extensive surgical intervention, if it reduces the food intake, may increase excitability. The second is that mild metabolic acidosis does not affect excitability; in experimental procedures in which a mild metabolic acidosis is incidentally produced, its effect can probably be disregarded. The third is that the evidence obtained on experimental animals does not agree with the clinical finding that starvation or a ketogenic diet reduces the incidence of seizures in epileptic patients. Not only is the electroshock threshold lowered by starvation, but the change in maximal seizure pattern is the direct opposite of that produced by clinically recognized antiepileptic drugs (Toman, Swinyard and Goodman, '46). In addition, our observations that the excitability may rise or fall in the face of similar degrees of ketosis show that ketosis itself is probably not responsible for the changes. In view of the unavoidable uncertainty of many clinical observations, the means by which starvation or ketogenic diets influence the course of epilepsy should be reëxamined.

SUMMARY

1. The electroshock threshold of rats increases as their body weight increases.

2. If the caloric intake is restricted, the electroshock threshold falls significantly, and it rises abruptly to normal when the restriction is removed.

3. Supplemental administration of vitamins of the B complex or of agar to rats having low thresholds as a result of restricted caloric intake has no effect upon the electroshock thresholds.

4. Supplemental administration of carbohydrate, fat or protein to rats having low electroshock thresholds as the result of caloric restriction raises the threshold.

5. Supplemental administration of fat to normal rats lowers the electroshock threshold.

6. The electroshock threshold may either rise or fall when mild metabolic acidosis is produced by starvation or fat feeding.

7. Mild metabolic acidosis produced by ammonium chloride has no effect upon the electroshock threshold, but extreme metabolic acidosis raises the threshold.

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BIOLOGICAL VALUES OF SIX PARTIALLY-PURIFIED PROTEINS [1]

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TWO FIGURES

(Received for publication February 13, 1948)

Because of conflicting data in the literature regarding the efficiency of utilization of proteins by different animal species, a coöperative endeavor by several different laboratories to resolve these discrepancies was proposed by the Bureau of Biological Research of Rutgers University. This laboratory was invited to assay the chosen proteins for their biological values by the maintenance method on human subjects.

The following proteins, all in the form of dry, granular or flour-like powders, were received directly from the committee, of which Dr. William H. Cole is chairman [2]: casein, egg albumen, whole egg, wheat gluten and peanut. The beef powder was received direct from Wilson and Company. All of the information available concerning the source and processing of the proteins was supplied by the committee.[3]

[1] This work was supported by a generous grant from the Williams and Waterman Fund of the Research Corporation of New York.

[2] The authors are greatly indebted to Dr. Cole and his committee for their patient and conscientious coöperation.

[3] This information will be found in the addendum.

EXPERIMENTAL

The diet squad

It is much more difficult to select a homogeneous group of human subjects, than of rats or even dogs. There was only 1 period of the year, namely, from June 1st to September, when the laboratory facilities, necessary for a nutritional investigation of this sort, could be freed entirely from their use in whole or in part by medical students. The selection of subjects therefore had to be made just when medical students were leaving. At other times we might have been able to select a group composed entirely of men or of women. Fortunately there was a sufficient number of men and women graduate and medical students remaining through the summer as assistants in this and other departments so that it was possible to assemble a squad of 11 composed of 5 women and 6 men. One man was excused on the 16th day and this left an equal number of each sex for the 4 determinative periods beginning June 21st, 1947. Preceding this date the subjects had been subsisting for 15 days on the 3 to 5% (of calories) egg diet which has been used so much in this laboratory both as a reference standard (Sumner, Pierce and Murlin, '38; Murlin, Nasset and Marsh, '38; Murlin, Marshall and Kochakian, '41) and as a recovery and stabilizing diet (Murlin, Edwards, Hawley and Clark, '46). This period of time was usefully employed in training the subjects to comply rigidly with a code of rules which each person signed, indicating his understanding and acceptance of them. On the whole they lived up to the rules. It is human to forget, and small lapses were excusable, if they were not serious, because we always had a sufficient number of days of collection so that omitting now and then a day from the data could not materially affect averages.

In confirmation of the impressions of the senior author as to the soundness of their health, all the original participants were given a physical examination by Dr. Lyman C. Boynton, part-time instructor in medicine and formerly a member of the department staff. All of them qualified.

Six of the original 11 served as subjects for the entire series of 15 experimental periods comprising a total of 85 days. Besides the first person excused on the 16th day, 4 others were excused on the 42nd, 47th, 51st and 61st days, respectively, because of persistent nausea or emesis, or both, for some days previously. Some of them, 1 young woman in particular, persisted in taking the diets longer than was good for them. None of these 4 suffered any serious harm,[4] however, and symptoms quickly disappeared when they returned to more normal diets. The very low protein diet which is called "no-protein" in this laboratory was the most difficult to endure; but the raw casein diet, because of its harsh, sandy roughness and utter lack of flavor, was almost equally so.

The characteristics of the diet squad members, which are of interest in this connection, are given in table 1.

After the 16th day, there were 5 men and 5 women until the 42nd day, i.e., through period VII, then 4 women and 5 men through period VIII, 4 of each sex through period IX, 4 women and 3 men through period XI, and thereafter 4 women and 5 men (see tables 3 and 4).

All members fell within the age limits of 21 to 30 years, inclusive. The weights given are the means of 2 weights obtained on the 2 days when each person's basal metabolism was determined — a period extending from August 8 to 28. The surface areas calculated from the formula of DuBois and DuBois are based on these mean weights and the original net heights. Likewise the caloric intakes are taken from the records of consumption at these times. With 1 exception (no. 11) there was no change in caloric intake during the last 4 periods. Adjustments of 50 to 100 cal. (rarely more; and always affecting the carbohydrate components only) were made from time to time as necessary to keep the subjects as nearly as possible at constant weight. The severely hot, humid weather prevailing in Rochester during a good share

[4] Their hemoglobin values, very kindly determined by Dr. E. E. Garber, were found to be within the normal range.

of the summer made it difficult to arrive at the exact requirements early or to maintain them evenly.

The last column in table 1 reflects also, to a considerable degree, the effect of warm unstimulating weather. The average of 32.5 cal. per square meter per hour for the women represents a deficit of 9.7% from the average for women between 20 and 30 years given by Boothby, Dunn and Berkson ('36), and the average of 35.9 a deficit of exactly 10% from the same standard for men within the same ages.

TABLE 1

The diet squad

NO.	SUBJECT	AGE	SEX	HEIGHT	NET WT.	SURFACE AREA	CALORIE INTAKE	BASAL MET. CAL/M²/HR.
				cm	*kg*	*m²*	*Per day*	
1	D.C.[1]	28	F	171	79.4	1.91	2600	29.4
2	E.R.[1]	21	M	175	72.0	1.86	3300	33.5
3	H.M.	27	M	163	54.5	1.57	2800	36.8
4	L.R.	30	M	185	70.3	1.94	3150	35.3
5	J.W.[1]	22	F	166	60.1	1.67	2200	33.2
7[2]	J.M.[1]	24	F	163	55.2	1.58	2650	31.1
8	A.F.	26	F	172	74.0	1.86	3000	35.4
9	W.K.[1]	22	M	181	79.1	1.99	3500	36.0
10	M.S.	26	F	156	54.5	1.53	2750	36.9
11	J.R.[1]	24	M	184	80.1	2.03	3550	34.7
12	M.E.[3]	27	M	176	73.0	1.88	3400	36.7
13	F.H.[3]	28	M	176	74.1	1.90	3400	38.1
14	H.G.[3]	22	F	157	44.9	1.42	2000	29.3
							Average for 6 women	32.5
							Average for 7 men	35.9

[1] These members served throughout the whole series.

[2] No. 6 dropped out earliest. The original number assigned each one was maintained in the interest of identification.

[3] These members were recruited in period X (see table 3).

The basal diet

The basal diet is illustrated in table 2 by that consumed by subject D.C. for period IX. The distribution of calories, (2.8% from protein, 62.2% from carbohydrate and 35% from fat) is fairly typical of the diets consumed by all sub-

jects when the protein constituent was of high biological value. (Observe the range of average test protein nitrogens ingested from wheat gluten to whole egg powder in figures 1 and 2.)

Butter was of the best creamery product [5] obtainable in Rochester. The pure fat was separated by melting and filtering in a hot funnel to remove traces of casein. The syrup

TABLE 2

The daily basal diet with egg albumen as the test protein (subject D.C., period IX)

FOOD ITEM	AMOUNT	NITROGEN
		gm
Butter (gm)	27	0.005
Syrup (gm)	75	0.019
Biscuit Mix with arrowroot starch (gm)	194	0.028
Lettuce, iceberg heart leaves (gm)	50	0.059
Carrot (gm)	25	0.019
Pickle (number)	1	0.007
French dressing (tablespoonful)	2	0.004
Orangeade, artificial (gm)	151	0.010
Applesauce (gm)	100	0.034
All vitamins (see text)	..	0.014
Coffee (number of cups)	1	0.047
Coca cola (bottles)	2	0.027
Total N		0.273
Less caffein N		0.074
		0.199
Egg albumen powder (21.7 gm)		2.66
Total available food N		2.86

Total calories: 2600.
Distribution of calories: protein 2.8%, carbohydrate 62.2%, fat 35%.

consisted of white corn syrup [6] to which some cane sugar and maple flavoring were added. It alternated with marmalade and pure fruit jellies as sources of flavor, and for spreads on the biscuits or "griddle cakes" made from the same mix. Arrowroot starch was chosen as the main component because of its very low nitrogen content The actual composition of

[5] "Land-O'-Lakes" brand.

[6] "Karo" brand.

the mix varied but little from the following formula: Arrowroot biscuits — each biscuit (or pancake or cookie depending upon amount of H_2O added), 18 gm; arrowroot starch, 8 gm; sugar, 8 gm; hydrogenated cottonseed oil [7], 1½ gm; baking powder, ½ gm; salt mix. The lettuce, grated carrot, with 3 seedless raisins, added for aesthetic effect, and a single sweet-sour pickle were served as a salad with the French dressing, composed of the purest cider vinegar and corn oil.[8]

The artificial orangeade was a mixture of white corn syrup, cane sugar, tartaric and citric acids in small amounts, orange extract flavoring and orange vegetable coloring. The apple sauce [9] had a carbohydrate content of 18%. There was some variation in the nitrogen content, denoting perhaps different mixtures of apples in different lots of 10-pound cans received.

Two different mixtures of B-complex vitamins designated *a* and *b* were used — one designed to supplement the vitamins contained in the natural food items, and the other designed not only to supplement, but also to stimulate and sustain appetite as the long series of periods wore on toward the end. These two expressed in milligrams per man per day are shown below.

	a	*b*
Riboflavin	0.0 mg	3.47 mg
Niacin	15.0 mg	20.5 mg
Para-aminobenzoic acid	6.3 mg	8.8 mg
Ca pantothenate	3.7 mg	6.4 mg
Pyridoxine · HCl	3.1 mg	4.0 mg
Thiamine · HCl	1.5 mg	2.2 mg
Inositol	100.0 mg	120.0 mg

All these constituents were dissolved in hot water in such quantity as to give the allotted proportions of the mixture *a* in 1 ml of solution when added to the artificial orangeade once a day. Riboflavin and choline were dissolved in 40% alcohol and dispensed in sufficient concentration to provide each subject 2.25 mg of the former and 15 mg of the latter in 1 ml

[7] Crisco.

[8] "Mazola" brand.

[9] "Blue Boy" brand made by the Haxton Co. of Oakfield, N. Y.

of solution, likewise added once a day to the orangeade, but taken at a different meal than the mixture *a*. In the *b* mixture, riboflavin was included, the whole being dissolved in water, to which about one-tenth of the volume of 95% alcohol was added. The solution was brought to boiling temperature, then cooled and bottled. Choline was dissolved separately in water in sufficient quantity to provide 48 mg of the substance in 1 ml of solution for each person, and always dispensed at a different meal than the *b* mixture.

One ascorbic acid tablet containing 100 mg and at first 1 capsule containing 1000 units of vitamin D and 5000 units of vitamin A were dispensed to each subject daily. Later the same unitage for the AD capsule was supplied by cod liver oil tablets,[10] thus avoiding nitrogen from the gelatin capsule.

The salt mixture employed in this study was, with very slight modifications, the same as that employed by Murlin, Edwards, Hawley and Clark ('46) and described on p. 535 of that communication. Coffee or tea was served at any meal desired, but was limited to 2 cups daily. It was prepared from soluble powder.[11] Tea bags were used in making tea, 1 bag to 2 cups. On account of their low N content, certain carbonated beverages [12] were permitted up to 2 bottles daily. The nitrogen in all of these beverages, called for convenience "caffein nitrogen," was determined frequently and always deducted from the nitrogen intake as having no food value. The corresponding amount for each subject was deducted from the urinary nitrogen, because it is usually excreted quantitatively.

The total nitrogen of the basal diet, less the caffein nitrogen in the case illustrated, was just short of 0.2 gm daily, but the average for the 8 members in this period was 0.184 gm or 5.7% of the total N. Averages for all the periods were: III, 0.29; IV, 0.35; V, 0.28; VI, 0.33; VII, 0.26; VIII, 0.23; IX, 0.18;

[10] From the White Laboratories, Newark, N. J.

[11] "Nescafe."

[12] "Coca Cola" and "Canada Dry" ginger ale.

X, 0.23; XI, 0.20; XII, 0.23; XIII, 0.23; XIV, 0.20; and XV, 0.21. The percentages of total food nitrogen ranged from 9% in period IV to 4.0% in period XIV.

Nitrogen determinations of all constituents of the basal diet as well as of the proteins fed were made in every period; but the 3 items making up the salad were for the most part homogenized together in the Waring blendor and determined as one to save time. Occasionally, the sum of the individual analyses was checked by taking aliquots of all items of a day's dietary, homogenizing them together and making but 1 analysis. The agreement was invariably quite close.

All analyses of food materials as well as of the urines and feces were made by the Macro-Kjeldahl method, employing sodium sulphate and copper sulphate in the proportion of 9:1 as catalyst and saturated boric acid, to which brom cresol green and methyl red in the proportion of 1:10 were added, in the receiving bottle for distillation.

Urines were collected in 24-hour periods for all days of the period but were analyzed, as a rule, on only the last 3 days. Feces were separated in periods by carmine or ferric oxide markers. Until the marker was obtained they were passed into white enameled pails; thereafter, directly into the 1-gallon "candy" collecting jars containing a few hundred ml of 5% sulfuric acid to which 10% of copper sulfate had been added. Special commodes designed to hold the jar or pail as well as a urine bottle were used.

Preparation of the food ("test" proteins)

In addition to the above-mentioned food items and their method of handling which comprised the "non protein" fraction of the diet, even greater precautions were necessary in the handling of the test proteins.

The fresh eggs were obtained from the same flock of birds fed on a constant diet. They were broken and approximately measured as to quantity (assuming 50 gm per egg) into the bowl of the electric mixer. They were then beaten at low speed

for 10 minutes, strained into a pitcher through a fine strainer and the desired amount poured with repeated stirrings directly into weighed beakers, on a balance. They were cooked by 3 methods. In periods 1, 2 and 7 they were scrambled or made into omelet on the stove top. In period 12 they were served as baked custard. They were so cooked in this period as to be in exact conformity with the other test proteins — in pyrex dishes placed in a pan of water and baked for 40 minutes at 350°F. In all periods where fresh egg was served 6 gm of the prescribed amount was added to the biscuits.

In general, the test protein was added to the biscuit mix and baked in pyrex dishes in the manner of the custard cooking. The biscuits were served to the subjects in the baking dish. Spatulas and rubber policemen, in addition to rinsing with water at the end, assured a quantitative transfer of protein.

It was not feasible, however, to add all of the egg albumen to the mix because the biscuits became too brittle and hard. A portion of this protein, therefore, was baked after mixing with water and served as baked egg white custard. Since it was impossible to prepare palatable biscuits incorporating the beef powder, some of it was served in soup and the remainder as a meat patty by the addition of different amounts of water. The cooking was done in a pan of water in the usual manner.

The raw casein obviously was not cooked. It was all mixed into a paste with water and eaten directly from the container with a spoon.

Table 3 exhibits the order of feeding the several test proteins, and the position of the fresh whole-egg and no-protein periods immediately following. Preparing the subjects by a high-value protein, from which there is little waste, for the no-protein period has a standardizing value which has been discussed by Murlin, Edwards, Hawley and Clark ('46). The table also gives the number of days for each period, influenced to some extent by the acceptability of the protein; the average nitrogen balance for the last 2 or 3 days of each; and

the number of subjects contributing to the balance. The reason for variation in the number of subjects has already been discussed.

The first no-protein period, it will be observed, ran for 7 days. Preceding it were 15 days — 3 before collections were started — on whole fresh egg. This protein was fed for the

TABLE 3

Order of feeding periods

NO.	PROTEIN	DAYS	AVERAGE N BALANCE	NUMBER OF SUBJECTS
			gm	
I	Fresh whole egg, begun June 6	6	— 0.28	11
II	Fresh whole egg, begun June 12	6	— 1.22	11
III	No protein	7	— 2.40	10
IV	1st casein powder	4	— 0.06	10
V	2nd casein powder (slightly higher N)	4	+ 0.36	10
VI	3rd raw casein	4	— 0.49	8
VII	Fresh whole egg	7	+ 0.13	10
VIII	No protein	5	— 2.68	8
IX	Egg albumen powder	5	— 0.15	8
X	1st whole egg powder	5	— 0.46	7 + 3[1]
XI	2nd whole egg powder (lower N)	5	— 0.61	7 + 3
XII	Fresh whole egg	6	+ 1.04	6 + 3
	(No protein for 3 new subjects)	6	— 2.58	
XIII	Wheat gluten	5	— 0.56	9
XIV	Peanut flour	6	— 0.14	9
XV	Beef powder, ended Aug. 30	6	+ 0.33	9

[1] Three new subjects joined the squad during this period but subsisted on fresh whole egg until the no-protein period for them (per. XII).

first 9 days at a level of 5% of the total calories, and in period II at 3%. The protein reserve of the subject thus was brought down gradually to near the very low point of the no-protein period. The lowest urinary nitrogen of the last 2 days was accepted as that for the endogenous level of excretion. The average for the 10 subjects was 2.01 gm. The average for 8 subjects in period VIII, which ran for only 5 days, was 1.97.

Including the 3 subjects whose no-protein ingestion coincided with that for period XII, the average for 9 persons for the last 3 periods was 1.86 gm (see line h of table 4). The relation of the individual endogenous urinary N to basal metabolism will be found in table 5.

RESULTS

The biological values

The data for estimation of the absorbed and the retained nitrogen values, from which the B.V.'s are calculated, are shown for the several proteins side by side in the order of feeding (table 4).

Three periods were devoted to the dry casein product. The method of preparation, as received from the coördinating committee, will be found in the addendum. It proved to be a very harsh, insipid powder and it required some experimentation, before the study started, to arrive at a satisfactory method of preparation, as has been related. The second period differs from the first in providing an average of almost 1 additional gram of ingested nitrogen for the squad of 10. This is the only reason assignable for the 0.3 gm higher average fecal excretion in this period. Obviously it is proportionally higher as shown by the lower percentage digestibility. The first period produced a very small fecal waste from the protein as measured by the excess over the no-protein period. A difference between the 2 periods of 0.67 gm in the calculated absorbed nitrogen resulted in only 0.26 mg more nitrogen in the urine. The no-protein urinary nitrogens being the same in the 2 periods, the waste nitrogens differ by the same amount, and these subtracted from the absorbed give retained nitrogens representing percentages of the absorbed (B.V.'s) that differ by only 1 unit. The raw casein was fed at a level midway between the 2 cooked casein periods. It proved not only less digestible but also less well utilized beyond the alimentary tract. The result was a biological value

TABLE 4

Biological values of 6 proteins

(Gm. N — Average of all members of squad)

	CASEIN 1	CASEIN 2	CASEIN RAW	EGG ALBUMEN	POWDERED WHOLE EGG	WHEAT GLUTEN	PEANUT	BEEF POWDER
(a) Test protein fecal N in gm	0.76	1.06	1.10	0.89	0.95	0.81	1.07	1.14
(b) No-protein fecal N in gm	0.72	0.72	0.72	0.94	0.94	1.01	1.01	1.01
(c) Test protein waste fecal N in gm (a–b)	0.04	0.34	0.38	— 0.05	0.01	— 0.20	0.06	0.13
(d) Test protein eaten N in gm	3.91	4.88	4.34	3.23	2.44	5.33	4.90	4.96
(e) Absorbed N (d–c)	3.87	4.54	3.96	3.28	2.43	5.53	4.84	4.68
(f) True digestibility $\frac{(e \times 100)}{d}$ in %	98	93	91	101	98	104	99	97
(g) Test protein urinary N (gm)	3.20	3.46	3.74	2.25	2.09	5.07	3.97	3.38
(h) No-protein urinary N (gm)	2.01	2.01	1.99	1.97	1.95	1.86	1.86	1.86
(i) Test protein waste urinary N in gm (g–h)	1.19	1.45	1.75	0.29	0.14	3.21	2.11	1.52
(j) Retained N (e–i)	2.68	3.09	2.21	2.99	2.29	2.32	2.73	3.16
(k) Biol. val. $\frac{(j \times 100)}{e}$	69	68	56	91	94	42	56	67
Number of subjects	10 5 ♂-5 ♀	10 5 ♂-5 ♀	9 5 ♂-4 ♀	8 4 ♂-4 ♀	7 3 ♂-4 ♀	9 5 ♂-4 ♀	9 5 ♂-4 ♀	9 5 ♂-4 ♀

of only 56 as compared with 69 and 68 for the 2 previous periods.

These results are shown graphically in figure 2 for the first cooked casein and the raw casein. Note the large amount of fecal and urinary waste nitrogens for the latter as indicated by the bracket and double arrow, respectively. Compare with egg albumen and whole egg on the same chart.

There is little more that needs to be said in explanation of table 4 or figures 1 and 2.

The first whole egg powder period (X) produced an average B.V. for the 7 subjects of only 75. It has been observed in other studies in this laboratory that a value like this is obtained once in a while, and it is believed such values are correct for the time and circumstances. But until an explanation can be given for these exceptional values they will be omitted. The one shown in table 4 and figure 2 is near the average of the many determinations in this laboratory on whole fresh egg. (Note 2 higher values for the fresh egg in figure 1 of the paper by Murlin et al. ['48].)

A question might be raised regarding the use of a negative difference between no-protein and food fecal nitrogens (table 4, egg albumen and wheat gluten) indicating higher than 100% digestibility and resulting in more absorbed than ingested nitrogen. However, it is not surprising that these 2 proteins should be more digestible than the small amount of protein contained in the no-protein diet, or that there should be less residual nitrogen from the intestinal secretions with highly digestible proteins. For these reasons, such differences have been used algebraically in this study, as shown.

Endogenous nitrogen of the urine

Since the work of Terroine and Sorg-Matter ('27) and of Smuts ('35), it has been maintained that mammalian species show a definite relationship between endogenous urine nitrogen and basal metabolism (Bricker, Mitchell and Kinsman, '45; Bricker and Mitchell, '47) such that 2.2 to 1.43 mg of the former correspond to 1 basal Calorie. In the 1946 study

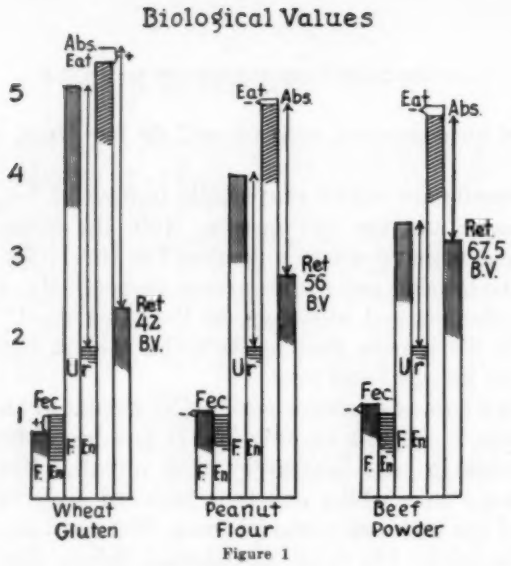

Figure 1

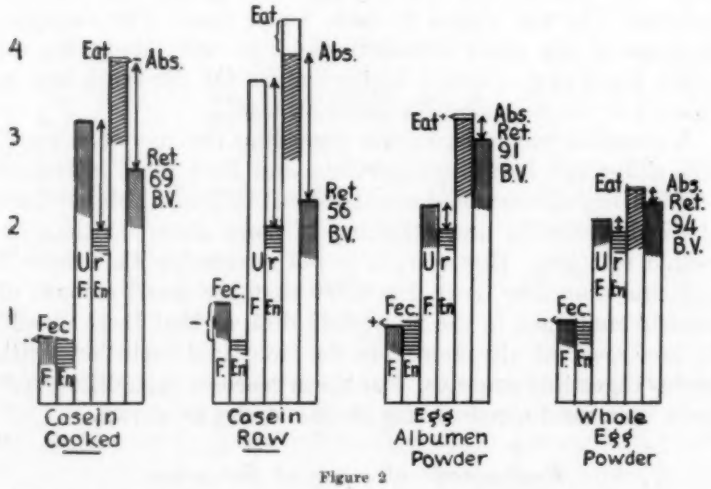

Figure 2

In each group of 6 bars:

1. The first pair of bars on the left refer to fecal nitrogen; the one marked "En" refers to the no-protein period and the one marked "F" refers to period in which the test protein was fed.

2. The center pair of bars represent the urinary excretion of nitrogen in protein and no-protein periods, respectively. The arrows represent waste nitrogen in excess of endogenous excretion.

3. The next to the last bar represents the nitrogen ingested and absorbed (the latter by diagonal hatching).

4. The last bar on the right represents the retained nitrogen. The figure alongside is the computed biological value (B.V.).

of endogenous nitrogen (Murlin, Edwards, Hawley and Clark), this ratio was found to be lower in 5 male subjects. Confirmation of this is seen in table 5, notwithstanding that the basal metabolisms of the 13 subjects averaged 10% below normal for their ages.

It would not be profitable at this time to compare the results regarding biological values with those in the literature. A

TABLE 5

Relation of endogenous N of urine to basal metabolism

SUBJECT	SEX	ENDOGENOUS URINARY N	BASAL METABOLISM	RATIO OF MG TO CAL.
		mg/24 hr.	*Cal./24 hr.*	
1	F	1910	1348	1.42
2	M	1960	1497	1.31
3	M	1760	1387	1.27
4	M	1990	1646	1.21
5	F	1510	1330	1.13
7	F	1310	1180	1.11
8	F	1800	1580	1.14
9	M	2450	1718	1.43
10	F	1570	1356	1.11
11	M	2610	1690	1.54
12	M	2075	1656	1.25
13	M	2170	1740	1.25
14	F	1240	998	1.24
			Average of 6 women	1.19
			Average of 7 men	1.32

comparison of all the new results with older ones should be made. So far as is known, this is the first of the several coöperative studies to be offered for publication. It will therefore be an inviting target, not to say a vulnerable one.

ADDENDUM

Egg albumen. The liquid egg white, either fresh or rapidly defrosted, is warmed to a specific temperature and inoculated with a culture to hasten fermentation. It is then allowed to ferment until all of the natural sugar is completely eliminated. After fermentation is complete, the liquid is chilled to stop fermentation.

It is held at reduced temperature until pumped to the dryer. The material is then converted to an aerated foam and dried continuously at an inlet air temperature not to exceed 150°F. (65.5°C.). The drying time is approximately 40 minutes. The product comes from the dryer in a very light fluffy form and is ground by merely rubbing it through a screen. This is, of course, done mechanically. The result is a product dried quickly at comparatively low temperatures, a product each particle of which has a very irregular surface which aids reconstitution with water.

Beef-benzol extracted muscle. Utility beef was used. The meat was trimmed as much as possible from fat and gristle and then ground. It was next heated to 180°F. (82.2°C.), spread on trays and dried in vacuum from the frozen state. It was then defatted with benzol and again dried in vacuum at low temperature to remove all solvent. The product was powdered sufficiently for incorporation into diets.

Whole egg. Frozen whole fresh eggs were used. They were desiccated and defatted by ethylene dichloride at temperatures below 70°C. Ethylene dichloride was the only reagent that came into contact with the product and it has been shown by Mitchell and others, who have examined other products, that this solvent in no way denatures the protein.

Peanut flour. Prepared by the McMath-Howard process, resulting in a partially defatted flour. The approximate analysis (in per cent) is protein 58.98, fat 9.69, N free extract 20.51, ash 3.84, crude fiber 2.54, moisture 4.44, phosphorus 0.565, calcium 0.065, magnesium 0.365 and iron 0.010. The vitamins (in μg/gm) were thiamine 6, riboflavin 3 and niacin 189.

Wheat gum gluten. Wheat flour agene-treated, of about 12% protein content, was selected. Cold water was added to form a dough in order to hydrate the gluten. More cold water was added to remove the starch leaving behind the gummy gluten. Five to 6 washings with water up to 90°F. were followed by 1 or 2 washings with water up to 170°F., the number of washings and the temperature depending upon the difficulty encountered in removing all the starch without losing the gummy properties of gluten. When the protein content was brought up to 80% on a dry basis, the material was dried on trays in a vacuum at 130°F. Each batch was specially handled in order to avoid denaturation of the protein (loss of gummy properties) as much as possible. Each batch varied from 4 to 5,000 pounds. It was not defatted. Actual temperature of gluten mass probably never exceeded 140°F., even when washed with water at 170°F.

Casein. The casein was prepared from a crude acid precipitated product. Purification consisted of repeated solution and reprecipita-

tion as well as thorough washing with dilute aqueous acid and salt solutions at temperatures of 40–55°C. No organic solvents were used. After drying in a stream of air at 60–70°C., the casein was ground in a hammer mill until 97% passed a 60-mesh sieve.

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CREATININE NITROGEN PERCENTAGE AS A CHECK ON THE BIOLOGICAL VALUES OF PROTEINS

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ONE FIGURE

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For several years past this laboratory has systematically used the creatinine nitrogen of the urine as a check on quantitative collections. Ever since Folin's ('05) famous paper on his new methods for urine analysis it has been known to be the most constant constituent on a creatine and creatinine free diet. In the course of the study described in the previous paper (Hawley, et al., '48) it had been observed that the creatinine nitrogen excretion expressed as milligrams daily was frequently very constant, not only for a single individual but also as an average of all squad members. Also that when expressed as percentage of the total nitrogen, there was a marked difference between high and low value proteins. This led to a study of the correlation between these 2 values not only from the most recent data available to us but also from earlier studies in this laboratory.

There are good reasons for expecting a high correlation between any constant component as a percentage of total nitrogen and the amount retained. Creatinine is without doubt the most typical endogenous product. Since biological values are calculated by subtracting the endogenous urinary nitrogen from the total urinary nitrogen after feeding the test protein,

the most genuinely endogenous constituent will, of necessity, vary in percentage of the total nitrogen inversely as this total nitrogen rises and falls with different kinds of food proteins. If the waste nitrogen of the food is high, this denotes a low retention and therefore low B.V., and produces a low creatinine N percentage; and vice versa.

A list of 14 periods were found, 9 from the study described in the preceding paper and 5 from the data of squads 8 and 9 of the paper by Murlin, Edwards, Hawley and Clark ('46, table 2) where the endogenous urinary nitrogens were unimpeachable and the biological values satisfactory. The correlation coefficient between the deviations from the mean values for biological value and for percentage of creatinine N in the urine was found to be 0.972. When the several means for the 2 variables obtained from the 14 periods for determination of biological values were plotted, the distribution seen in figure 1 was obtained. The ideal regression line was fitted to the data by the method of least squares or errors of estimate by the equation $E = \bar{y} + \frac{S\,x\,y}{S\,x^2}(X - \bar{x})$, according to Snedecor ('37). E is the estimate of the ordinate (biological value) for any chosen value of the abscissa X (creatinine percentage), $\bar{x}$ is the mean of the determined values, and $\bar{y}$ is the mean of the ordinates. Sxy is the sum of the products of the deviations from the means, and Sx^2 is the sum of the squares of the deviations from the mean creatinine percentage.

The position of the conjunction of the 2 means, $\bar{x}$ and $\bar{y}$, is at the central X. The value of E for 16.4% creatinine nitrogen is found by the computation to be at the position on the line of the lower X, and the value of E for 21% creatinine N is at the position of the upper X. The regression line drawn through these 3 points describes the average rate of change in E for a unit change in X.

From the first 4 points located on the plotting, it was possible to predict approximately the biological value of wheat gluten from the average creatinine nitrogen percentages. From the average of these percentages for the first day of analysis when peanut was the test protein, its biological value

was predicted quite accurately, as determined later when all analyses were at hand. It will, of course, require many more data before the relationship can be established with finality.

The conditions which will produce still more constant creatinine excretions have yet to be worked out by much experimentation. The photoelectric colorimeter by which all

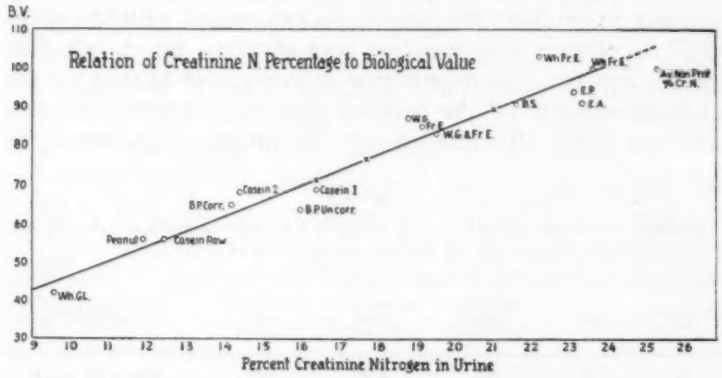

Fig. 1 Wh.Gl. denotes wheat gluten, B.P. corr., and B.P. uncorr., beef protein corrected and uncorrected for the excess creatinine nitrogen in the urine of the beef period over that of the peanut period which just preceded it. The 3 casein periods of the tests described in the current study are indicated. E.P., E.A. and Wh.Fr.E. (lower) indicate the coordinate points for whole egg powder, egg albumen powder and for whole fresh egg protein of periods XI, IX and VII. W.G., Fr.E., and W.G. & Fr.E., are points taken from the values found for wheat germ, fresh whole egg, and wheat germ 50% and fresh whole egg 50% — all from the data of squad 9 of the 1946 study. Wh.Fr.E. (upper value B.V. 104) and B.S. are for fresh whole egg and beef steak as found from squad 8 in the same study. B.P. uncorr. is not counted in the correlation.

creatinine determinations have been made in this laboratory for many years, is so much more dependable than the old Duboscq method that older values would scarcely be acceptable.

DuBos and Miller ('37) have shown by their specific enzymatic determination that creatinine of normal and even of nephritic urines constitutes nearly 100% of the chromogenic

substance contained. Therefore, unless some kind of stable artificial coloring matter from the food should occur in the urine, the excretion through normal kidneys certainly would reflect the rate of endogenous metabolism from a diet containing no creatine or creatinine.

If, as in the case of the beef powder (B.P. on the graph), the creatinine content of the food can be determined, a correction can be applied. The powder was extracted with hot water until no chromogenic reaction was obtained by the alkaline picrate reagent. The result was an average of 149 mg for the average amount of the powder ingested. Apparently this was not quite all excreted for the difference between the

TABLE 1

Average creatinine excretion of 6 subjects who were longest on the diets. All values are in milligrams of creatinine N daily

SUBJECT NO.	SEX	AVERAGE OF FIRST 10 DAYS	AVERAGE OF NEXT 20 DAYS	AVERAGE OF LAST 15 DAYS	VARIABILITY OVER ALL IN %
1	F	505	458	451	$466 \pm 27 \pm 5.8$
2	M	553	537	533	$535 \pm 26 \pm 4.8$
5	F	396	359	342	$363 \pm 21 \pm 5.8$
7	F	390	360	347	$363 \pm 17 \pm 4.7$
9	M	642	608	605	$620 \pm 20 \pm 3.2$
11	M	659	651	616	$639 \pm 32 \pm 5.0$

creatinine of the beef period and of the peanut period was only 114 mg. Peanut gave no chromogenic reaction. Consequently, the correction exhibited in the graph was based on the average difference for all the 9 members of the squad.

The variability in creatinine nitrogen, as shown by the 6 members who served throughout the entire series of experiments, is shown in table 1. There was, with 1 exception, a rather large decrease from the first 10 days and the next 20 days but (again the same exception) only a slight change to the last 15 days.

The variability for the entire 45 days on which urinary analyses were made is only from 3.2% for subject 9 to 5.8%

for subjects 1 and 5. Such variations do not invalidate the correlation shown in the graph.

It is of interest to note that the creatinine percentage of the total N on the no-protein diets used in this laboratory is 25.3, the mean of 39 determinations. This value is placed at the 100% line in the graph, not to indicate a biological value, for that obviously is not possible for the very small nitrogen intake, but to call attention to the fact that some constituent of the diet, possibly carbohydrate, exercises a sparing action on creatinine. The regression line is dotted to the position which a B.V. corresponding to 25.3% would occupy. Without doubt, the percentage would be higher in starvation.

It would seem that the biological value of any mixed diet could be obtained approximately by feeding it at a level low enough to obtain a minus N balance, and determining the creatinine nitrogen percentage. This possibility is now under investigation.

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FOOD AND WATER ECONOMY OF THE YOUNG RAT DURING CHRONIC STARVATION AND RECOVERY

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TWO FIGURES

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Only a few scattered and unrelated conclusions have been made pertaining to the alterations in food utilization and water consumption during chronic starvation and recovery. Thompson and Mendel ('18) have shown that in mice the weight gained during refeeding of undernourished and stunted animals was greater in proportion to the food eaten than that gained by the controls. Keys et al. ('46) found that in adult men the weight recovery was greatest on high caloric diets. It is stated by Morgulis ('23) that the gross gain in weight may exceed the quantity of food consumed due to the fact that during re-alimentation the organism retains much water. Benedict ('07) and Keys et al. ('46) also found that water is retained during starvation, and noted in this connection an increase in water intake and water excretion.

The studies made in this experiment to ascertain the influence of chronic starvation and recovery on food and water economy were approached in the following ways: (a) by determining the amount of food and water required each day to maintain a constant body weight during chronic starvation, (b) by observing the course of recovery of body weight both as to the amount of food and water consumed and the time required, and (c) by determining the degree of absorption of

food materials in chronically starved rats as compared to that in normal rats.

METHODS

The investigations made in this work were arranged in 2 separate experiments. In both experiments young male albino rats, 30 days of age and about 50 gm in weight, were employed in groups of 50, each group constituting an experimental unit. The rats were placed in individual cages and were underfed by restriction to a constant daily body weight ration of a qualitatively balanced diet. The synthetic stock food employed is shown in table 1.

TABLE 1

Composition of synthetic stock ration in amount per gram

Protein	240.0 mg	Thiamine	0.005 mg
Carbohydrate	450.0 mg	Pantothenic acid	0.012 mg
Fat	45.0 mg	Niacin	0.0056 mg
Fiber	40.0 mg	Riboflavin	4.0 μg
Ash	100.0 mg	Vitamin D	60.0 chick units
Water	100.0 mg	Vitamin A	600.0 U.S.P. units
Salt	2.5 mg	Vitamins C, E and K present in unmeasured amounts	
Iron oxide	2.5 mg		

Adjustments of the amount of food allowed daily were made with each rat according to the loss or gain in body weight. At the end of the selected periods of underfeeding the animals were given full and adequate amounts of the above ration for 2 different periods of time. In experiment I, 50 rats were underfed for 30 days and refed for 35 days; in experiment II, 50 rats were underfed for 90 days and refed for 60 days. In addition to these groups of refed rats, a group of fully fed controls was observed during the recovery period of experiment I; the animals of this normal group were of the same size as the experimental animals at the end of the starvation period.

Daily food consumption during recovery was determined by placing a given quantity in the cages and weighing the dry residue 24 hours later. Water consumption was determined by

recording the number of times cage bottles were filled. Wax lines were marked on the bottles so that refilling represented a certain number of milliliters. The animals were weighed daily in experiment I and weekly in experiment II. All weights were taken at the same time each day and on the same day each week.

There was reason to believe that food-feces ratios would throw light on the problem of food economy. It is conceivable, for instance, that an increase in the ability of the digestive system to absorb usable material from ingested food could contribute to body weight gain. Ficker ('06) speaks of the increased permeability of the epithelial lining of the digestive tract to microorganisms as a result of starvation. Brooks, Marine and Lambert ('46) employed food-feces ratios with success in determining the efficiency of digestive functions in experimental obesity. Methods similar to those employed by these workers were used in this experiment. Food intake was determined for a period of 5 days by weighing the dry residue in the cages of 10 control rats and in those of 10 experimental rats which had been starved for 60 days. The dry weight of the feces was determined by collecting and desiccating the feces from both the control and experimental groups over this same period.

RESULTS AND DISCUSSION

The average daily food rations necessary to maintain a constant body weight during starvation are shown in figure 1. Although all rats in the preliminary experiments were allowed an identical daily ration, there was considerable variation in the ability to withstand sudden caloric restriction. Rats that survived the first few days of restricted food intake lived throughout the starvation periods. Gradual reduction in the daily ration in early starvation eliminated mortalities and made it possible later to maintain all animals on the same amount of food. Mortalities which nearly always occurred at the beginning of starvation in the preliminary experiments were eliminated entirely in the final studies by individual

food and body weight adjustments. The animals in chronic starvation required progressively less food to maintain body weight as the underfeeding was continued. These results may be viewed as an adaptation or acclimatization. The probable mechanism here is twofold: (a) the increased absorption efficiency of the digestive tract of the starved rats, as appears below in the results of this experiment and (b) the reduction in metabolic rate which resulted from starvation (Quimby and Phillips, '47). Both of these effects would improve the food economy of the animal, the former actually providing

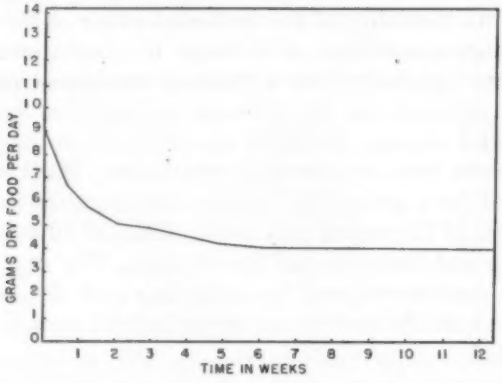

Fig. 1 The amount of food required daily during chronic starvation to maintain a constant body weight in young rats. The curve represents an average of 50 animals.

more energy for the tissues, the latter reducing the basal energy demand.

The average total water intake, including that consumed with the food, for the starved and fully fed normal rats during a 30-day period is presented in table 2. The amount of dry food consumed by these 2 groups over this same period and the food-water ratio are also shown. These data show that although there was greater water intake in proportion to the amount of food consumed on the part of the starved rats, the total water consumption was not great. This finding

stands in contrast to (1) that of Benedict ('07) who observed that starving human subjects drank enormous volumes of water, and (2) that reported by Keys et al. ('46) who noted that semi-starved men more than doubled their water intake. It appears, therefore, that the water retention noted by Benedict in starving humans and the excessive diuresis observed by Keys et al. in semi-starved men were not typical of the chronically starved young rats of this investigation.

The relationship between the weight of dry food consumed and the weight of dried feces is expressed in table 2. These figures show that there was greater food absorption in the starved rats than in those which were fully fed. This fact suggests either a decrease in peristalsis or an increase in the permeability of the epithelial lining of the digestive tract.

TABLE 2

Food-water and food-feces ratios of chronically starved and fully fed rats

EXPERIMENTAL GROUP	FOOD CONSUMPTION	WATER INTAKE	FOOD-WATER RATIO	WEIGHT OF DRY FECES PER DAY	WEIGHT OF DRY FOOD PER DAY	FOOD-FECES RATIO
	gm	*ml*		*gm*	*gm*	
Starved	150	373	1 : 2.5	1.14	4.5	1 : .25
Fully fed	520	984	1 : 1.9	5.20	17.1	1 : .34

The daily food consumption of starved rats during the first 20 days of recovery as compared to that of normal rats of the same size is presented in figure 2. Anorexia did not develop in any of the starved animals; food upon re-alimentation was accepted with vigor. However, during the first 10 days of recovery feeding the rats which had been chronically starved exhibited less appetite than normal rats of the same size. The only exception to this occurred on the very first day that food was allowed ad libitum. After the 12th day of re-alimentation the rats recovering from chronic starvation had a greater appetite than the controls and ultimately accumulated a greater total food consumption (table 4 and fig. 2). No deleterious effects of the ingestion of large amounts of food on this first day of recovery were noted in these rats,

such as were observed by Benedict ('07) and Keys et al. ('46) in humans. The decreased food intake on the second day may indicate some reaction or illness of the animal, or may have been simply an appetite reduction due to the indulgence of the first day. The latter is the more likely explanation since it was observed that in both normal and refed rats there was a tendency for food consumption to alternate between high and low on successive days.

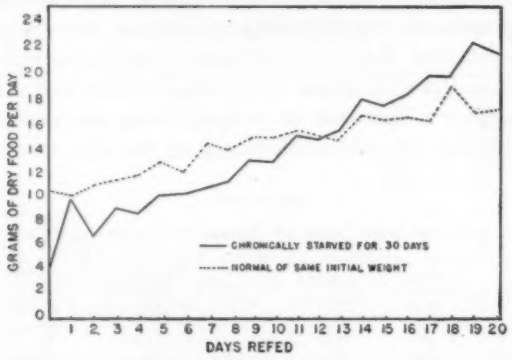

Fig. 2 Comparison of daily food consumption of refed starved rats with normal rats of the same initial weight. Each line on the graph represents the average of 10 rats.

The relationship between food consumption and weight gain from the 60-day recovery period of experiment II is summarized in table 3. It can be seen from these data that the greater caloric intake results in an increased weight recovery. Similarly Keys et al. ('46) noted that the weight recovery in adult men who had been chronically starved was greatest on high caloric diets. A gross gain in weight exceeding the weight of consumed food, as noted by Morgulis ('23) on humans, was not observed in this investigation with rats.

The effects of re-alimentation on food consumption, food utilization, weight gain, and water intake are summarized in table 4, together with similar data for the controls. Upon

refeeding, the rats which had been starved exhibited a remarkably rapid rate of growth. This is shown by the data for the rats of experiment I who after 35 days of refeeding had an average weight of 246 gm compared with the normal rats of

TABLE 3

Food consumption, weight gain, and food utilization during recovery from chronic starvation

NUMBER OF ANIMALS	AVERAGE TOTAL FOOD CONSUMPTION	AVERAGE TOTAL WEIGHT GAIN	PER CENT FOOD UTILIZATION [1]
	gm	*gm*	
12	808.3 (714–859)	174.4 (132–200)	21.4
16	959.6 (905–1059)	216.7 (204–239)	21.5
8	1218.7 (1105–1346)	271.0 (251–289)	22.2

[1] $\frac{\text{Grams increase in weight}}{\text{Grams of food consumed}}$

TABLE 4

Weight gain, food consumption, food utilization and water intake of normal and refed rats

NO. OF RATS IN GROUP	INITIAL WEIGHT	FINAL WEIGHT	WEIGHT GAIN	GAIN	RECOVERY (OF NORMAL)	FOOD CONSUMPTION	PER CENT OF FOOD UTILIZATION [1]	WATER INTAKE
	gm	*gm*	*gm*	%	%	*gm*		*ml*
Experiment I — Chronically starved for 30 days, and refed for 35 days								
50	58.3	246.1	187.9	321.8	83.7	633.1	29.9	852.8
Fully fed control, of same initial weight as experiment I								
10	60.5	195.2	134.7	222.2	63.3	576.0	23.4	1151.0
Experiment II — Chronically starved for 90 days and refed for 60 days								
50	60.3	267.1	206.7	340.8	75.4	965.7	21.3	1834.0

[1] $\frac{\text{Grams increase in weight}}{\text{Grams of food consumed}}$

the same initial weight whose average weight after 35 days of refeeding was only 191 gm. This stimulation of growth by starvation may be further emphasized by comparison with the growth rate found by Bryan and Gaiser ('32) in normal rats fed a special growth ration. Fed on what they call

"Mendel's special growth diet" but which is really a ration devised by Smith and Bing ('28), the normal rats observed by these workers required 38 days to grow from 60 to 200 gm. The chronically starved rats of this present experiment required only 22 and 30 days to grow through this identical range of body weight after being starved 30 and 90 days, respectively. Kopec and Latyszewski ('32) interpret this increased growth rate of refed animals as simply indicating a stimulating effect of starvation on growth. Jackson ('25) states that the rapid growth of the body following various periods of inanition may be due to the embryonic condition to which the cells are reduced. The extremely high metabolic rates during recovery found in studies related to the present investigation (Quimby and Phillips, '47) may well support this concept of embryonic cell growth. However, the results of the present study indicate that growth stimulation following starvation may be related to the greater food utilization efficiency which starvation effects. While to some extent the enhanced growth may have been contributed to by slightly greater food consumption, it can be seen in table 3 that the group which had been starved had a food utilization of 29.9% as compared to 23.4% for the controls. This increased food utilization efficiency may have been due to the increased intestinal absorption which was incurred during chronic starvation. Results similar to this have been obtained on mice by Thompson and Mendel ('18) who concluded that the weight gained by the stunted animals in proportion to the food eaten was greater than that gained by the controls.

The recovery of a normal body weight by the stunted animals was, of course, somewhat proportional to the duration of refeeding. Prolonged inanition increased the time for recovery, however. The mean weight of rats starved for 30 days and refed for 35 days was 246 gm, while that of rats starved for 90 days and refed for 35 days was only 220 gm. In other words, after equal periods of refeeding the rats which had been held in a state of chronic inanition for a prolonged period recovered considerably less in body weight than rats which

had been starved for only a short time. It may be concluded, therefore, that while short periods of semi-starvation stimulate growth, prolonged starvation tends to reduce this effect. Whether this difference in growth rate following short and long periods of chronic starvation is a reflection of the differences in ages of the animals, or whether it is a reflection of fundamental tissue changes associated with the malnutrition has not been determined.

SUMMARY

1. There was considerable variation in the ability of the rats to withstand sudden caloric restriction. Gradual reduction in the daily ration in early starvation eliminated mortalities and cognizance of adaptive differences carried out by individual weight losses and food ration adjustments made it possible later to maintain all animals on the same amount of food. The quantity of food necessary to maintain a constant body weight became less as underfeeding was continued.

2. The amount of water consumed by the starved rats was less than that consumed by fully fed rats of the same initial size, but was greater in proportion to the food intake.

3. Chronically starved rats absorbed more material from the food than did normal rats.

4. Anorexia did not develop as a result of chronic starvation, although during early re-alimentation the underfed rats consumed on the average slightly less food than normal rats of the same initial weight. The starved animals when refed accepted food with vigor, and the ingestion of large amounts appeared to have no ill-effects.

5. Animals consuming large amounts of food during re-alimentation exhibited a greater weight gain than those with lesser appetites.

6. Rats underfed for 30 days had a greater growth rate and food efficiency than normal rats of the same size. This effect was not seen in rats of the more prolonged starvation period of 90 days.

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